UNIVERSITY OF HAWAIʻI AT MĀNOA

MASTERS THESIS

PATTERNS AND EFFECTS OF DIRECT CONTACT BETWEEN CORAL AND MACROALGAE ON SHALLOW REEFS AROUND OʻAHU, HAWAIʻI

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Abstract

O‘ahu’s reefs are vulnerable to increased macroalgal growth, which can have severe negative effects on reef corals. While experiments investigating these interactions have increased in recent years, most are short (less than 20 days) and naturally-occurring coral-algal interactions are rarely surveyed.

Surveys of nearshore O‘ahu reefs seeking to quantify naturally-occurring coral-algal interactions for the first time found significant differences in the number and severity of interactions experienced by different coral species as well as between sites. This research provides a valuable baseline and springboard for future research.

A three-month experiment exposed *Porites lobata* coral to mats of the invasive *Gracilaria salicornia* and observed higher prevalence and severity of bleaching, reduced photosynthetic pigment concentrations, and altered lipid content in treatment corals. However, differences in physiological metrics over time suggest that the response may be more immediate and acute than chronic.

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Chapter 1

Introduction

1.1 Algae on Coral Reefs

Tropical coral reefs have often been compared to tropical rain forests not only because both support high levels of biodiversity, but also because both exist in a seeming paradox - abundant and diverse life in the midst of what appears to be a nutrient desert (Connell, 1978). The poor soil of the rainforest and the clear oligotrophic water of coral reefs belie the high productivity of each system because both rely on high turnover of nutrients rather than standing surpluses.

Surveys on the most pristine and least impacted coral reefs on the planet have demonstrated the inverted food pyramid proposed by Odum and Odum (1955), where the majority of standing biomass is in large-bodied apex predators and the least standing biomass is in primary producers such as algae (Pandolfi, 2003; Steneck and Sala, 2005; Sandin et al., 2008; Bruno et al., 2014). This system works because on pristine reefs the fast growth of algae is matched by the grazing pressure of herbivores, which eagerly seek out and bite off any algae tips that extend out of crevices between coral.

Benthic marine algae are an important, productive, beneficial component of a healthy coral reef system (Fong and Paul, 2011). Many coral reef species are tightly associated with macroalgae, including mesograzers, epiphytes, and juveniles of some fish and invertebrates (Hay, 1997; **?**; Karez et al., 2000; Angel et al., 2002; Ceccarelli et al., 2005). However, increased environmental and anthropogenic stressors in recent decades (Bellwood et al., 2004; Wolanski et al., 2004; Pandolfi et al., 2005; Bruno and Selig, 2007; Bruno, 2013) have contributed to widespread coral degradation, allowing algal growth to surpass the rate of herbivory, leading to what is known as a phase shift (Done, 1992; Littler et al., 1993; Steven and Larkum, 1993; Stimson et al., 2001; Smith et al., 2001).

1.2 Phase Shifts

The most well-documented phase shift occurred in Jamaica in 1984 after a sequence of natural and anthropogenic events described by Hughes (1994), leading to a decrease from over 50% coral cover to less than 5%. This classic paper demonstrates the synergistic effects of multiple stressors such as overfishing and damage from natural disasters. Macroalgae blooms on Jamaican reefs and the resulting shift to algal dominance began immediately after the massive *Diadema* (sea urchin) die-off that swept through the Caribbean from 1982-1984, even though the reef had survived through a massive reduction in herbivorous fish biomass and diversity due to overfishing as well as the destructive effects of multiple hurricanes.

The occurrence of this and other phase shifts around the world (Ledlie et al., 2007; Maliao et al., 2008; Lesser and Slattery, 2011) have sparked intense study on the relative importance of bottom-up (nutrient, see Lapointe (1997); McCook (1999)) versus top-down (predation/herbivory, see Hughes et al. (2007); McManus et al. (2000)) controls of shifts, with many studies showing a complicated interplay between the two mechanisms (McManus and Polsenberg, 2004; Littler et al., 2006; Smith et al., 2010). It has also been suggested that large-scale disturbances, such as El Niño-related bleaching events, are what tip the scale and allow phase shifts to occur on previously healthy reefs (Sergeeva et al., 2007; Bruno et al., 2014).

This brings up the important role of reef resilience. Case studies of phase shifts like the one in Jamaica demonstrate that independent disturbances can move a reef towards a position where it is less resilient to stressors and more vulnerable to a phase shift. Noted coral reef ecologists have also reasoned that reefs differ in intrinsic resilience, with Pacific reefs being much less prone to large scale disturbance than Caribbean reefs, and even going so far as to suggest that alternate stable states of algae dominance are not possible for some reefs (Roff and Mumby, 2012; Mumby et al., 2015).

1.2.1 Algae Blooms and Introductions in Hawaii

O'ahu, Hawai'i has been the site of at least 19 intentional or unintentional introductions of non-native macroalgae since 1950, five of which have become successful invaders (Smith et al., 2002). These algal blooms and invasions have caused damage to native species and ecosystems, with some reefs around the island having over 50% cover of alien frondose algae (Smith, 2003; Friedlander et al., 2008).

The most abundant and common alien invasive macroalga in Hawai'i is the red alga *Acanthophora spicifera*, which was introduced from Guam 1n 1950 and is now found on every main Hawaiian island (Doty, 1961; Smith et al., 2002). This alga has thin, fragile thalli that easily break off and spread through vegetative reproduction, out-competing native macroalgae and taking over nearshore ecosystems (Russel and Balaz, 1994; Weijerman et al., 2008). *Hypnea musciformis*, the second most abundant alien macroalgae in Hawai'i, was orginally introduced from Florida in 1974 for aquaculture of kappa carrageenan (Smith et al., 2002). The specific morphology of this alga, with hook-like endings at the tips of the thalli, makes it exceptionally successful at vegetative reproduction (Smith et al., 2002).

Two invasive mat-forming algae were introduced to Kāne'ohe Bay and Waikīkī, O'ahu between 1970-1978 (Rodgers and Cox, 1999; Ask et al., 2003; Smith et al., 2004): The *Eucheuma denticulatum/Kappaphycus spp.* species complex as well as *Gracilaria salicornia*, which is locally known as "gorilla ogo". Both are reported to be very fast growing, able to cover reef areas at a rate of 250-280 meters per year (Rodgers and Cox, 1999), and form thick, complex mats that can reach densities of 30 kg/m^2 and grow by as much as 10% per day (Smith et al., 2004; Conklin and Smith, 2005). These algal mats are not preferred by reef herbivores (Stimson and Conklin, 2008), and have been implicated in causing coral death through smothering (Russel, 1983; Woo et al., 2002; Conklin and Smith, 2005; Godwin et al., 2006). While *G. salicornia* has expanded its range over 5km from its site of introduction, *Kappaphycus spp.* has not been widely reported

outside of Kāneʻohe Bay, Oʻahu (Smith et al., 2002; Bahr et al., 2015), although it has been observed just North of the bay, at Hauula reef (Celia Smith, personal communication).

Avrainvillea amadelpha, commonly known as "leather mud weed" is a green siphonous macroalga that was first noted in Hawaiʻi in 1980, and has since overtaken much of Maunalua Bay on the south shore of Oʻahu. The thick holdfasts of this alga trap sediment while the leathery fronds slow water movement, increasing residence time and sediment retention in the bay (Stamski and Field, 2006; Wolanski et al., 2009). Changes in the physical environment of the bay from stream channelization and urbanization, coupled with the growth and spread of invasive algae have caused native seagrass (*Halophila hawaiiana*) and coral species to be competitively inferior, with collapses of coral populations observed on the west side of the bay (Unabia, 1984; Wolanski et al., 2009). Algae removal efforts (locally known as "huki" or "pulls") have been at least temporarily successful in clearing large areas of the bay that were once dominated by the invasive *A. amadelpha*, and groups have begun experimental outplanting of seaweed and native "limu" (macroalgae) species to restore the nearshore community of the bay (Kittinger et al., 2016).

Native species of macroalgae can also form large-scale blooms in response to environmental changes (Russell 1987, 1999, Stimson et al. 1996). *Ulva fasciata* and *Cladophora sericea*, two species of green algae, have both been seen to bloom in large numbers on reefs around Maui, Hawaiʻi (Smith et al., 2002). Kāneʻohe Bay on Oʻahu, Hawaiʻi, is the site of a well-studied bloom of the green "bubble alga," *Dictyosphaeria cavernosa*, due to eutrophication caused by sewage outfalls into the bay between 1940-1970's (Smith et al., 1981; Hunter and Evans, 1995; Stimson et al., 2001). *D. cavernosa* overgrew and eliminated corals, taking over large areas of patch reef in the bay (Smith et al., 1981; Bahr et al., 2015). After major sewage outfalls were diverted in 1977-1978, nutrient levels dropped and *D. cavernosa* abundance was reduced by around 75% (Smith et al., 1981; Hunter and Evans, 1995), representing what has been called a "reverse phase shift" (Stimson et al., 2001; Stimson and Conklin, 2008; Bahr et al., 2015).

1.3 Coral-Algae Interactions

In Hawaii and elsewhere, cover of benthic macroalgae on coral reefs is increasing in response to increased nutrients, decreased herbivory, and other anthropogenic effects (Hatcher and Larkum, 1983; Littler and Littler, 1984; Steven and Larkum, 1993; Stimson et al., 2001; Smith et al., 2001, 2002). This increased prevalence of macroalgae on reefs necessarily brings coral and algae into closer and more frequent contact as both struggle to secure space on hard substrates. An upward trend in the number of coral-algae interaction research papers in recent years (Barott and Rohwer, 2012) suggests these interactions are becoming more noticeable if not more impactful.

Laboratory exposure experiments as well as *in situ* algae exposure and removal field experiments have demonstrated that some species of benthic algae can have direct negative impacts on corals in the vicinity through physical alteration of the environment, disease transmission, altered microbial activity, and allelopathy (McCook et al., 2001; Nugues et al., 2004).

1.3.1 Physical Impacts

A high standing biomass of algae can alter the physical and chemical environment around it in ways that can be directly detrimental to corals growing in close proximity. These include shading, abrasion, reduced water low, sedimentation, and changes in water chemistry due to photosynthetic activity (Hauri et al., 2010; Martinez et al., 2012). Mats of *Gracilaria salicornia* in Hawai'i were found to decrease photosynthetically active radiation to understory corals by as much as 99% (Martinez et al., 2012). Dense canopies and mats of algae reduce water flow and thus increase concentrations of dissolved organic and inorganic carbon (DOC/DIC). This decreased flushing also increases sedimentation, with up to twice as much sediment settling under *Gracilaria salicornia* mats than at control sites (Martinez et al., 2012; Hauri et al., 2010; Stamski and Field, 2006).

Algae also have direct physical effects on coral, which may depend largely on algal functional groups. Larger, canopy-forming species such as *Sargassum spp.* shade and abrade coral colonies through whip-like movements, while smaller, mat-forming species such as *Gracilaria spp.* can grow over top of coral colonies, thereby smothering them (McCook et al., 2001). Hauri et al. (2010) found that increased algal biomass significantly strengthened negative effects on corals for mat-forming but not canopy-forming species.

1.3.2 Disease Transmission

It has been observed that benthic algae biomass is highly correlated with coral disease prevalence, suggesting that algae may act as pathogen reservoirs on the reef. Nugues et al. (2004) showed that direct contact with *Halimeda opuntia* was enough to trigger the onset of an aggressive Caribbean coral disease known as White Plague II in *Orbicella faveolata* - a dominant reef-building coral. Though they made the concession that the pathogen (*Aurantimonas coralicida*) may not have been transmitted by the algae itself, but by an organism associated with it (such as corallivorous invertebrates), the correlation has held up through further studies, prompting Sweet et al. (2013) to call benthic algae "common reservoirs" for potential coral pathogens.

While benthic algae are able to directly transmit pathogens to corals through physical contact, it is likely that the mere presence of a pathogen reservoir in contact with coral is not enough to trigger the onset of disease without pre-existing stress or damage to the coral animal (Sweet et al., 2013). Lesser et al. (2007) suggested that all incidences of coral disease are opportunistic infections occurring after exposure to physiological stress such as temperature stress or mechanical abrasion.

1.3.3 Microbial Activity

In recent years the concept of the coral holobiont has been expanded to include not only the coral animal and dinoflagellate *Symbiodinium*, but also the associated microbiota of the surface mucus layer (SML) (Johnston and Rohwer, 2007; Rohwer and Kelley, 2004). Coral-associated microbes (both bacteria and

viruses) are thought to have a major role in protection from infection (Ritchie, 2006; Rosenberg et al., 2007) and my play a role in immunity (Reshef et al., 2006), though the full extent of their role in the holobiont is largely unknown (Morrow et al., 2012b). This assemblage has been shown to be somewhat species-specific (Rohwer et al., 2002; Kvennefors et al., 2010; Morrow et al., 2012a), but has also been seen to adapt to changes in location and environmental conditions (Wommack et al., 1999). Shifts in the microbial assemblage can increase coral susceptibility to pathogens by decreasing beneficial species or increasing potential pathogens (Morrow et al., 2012a).

Benthic algae may influence the make-up and activity of the coral SML microbiota by introducing novel microbes from their own surface microbial assemblage or by stimulating microbial activity with leached photosynthates (Steinberg et al., 2002; Smith et al., 2006; Kline et al., 2006; Dinsdale et al., 2008).

1.3.4 Allelopathy

Benthic marine algae are known to produce varying amounts of secondary metabolites with varying potencies of anti-herbivore and anti-fouling compounds (Schmitt et al., 1995; Cronin and Hay, 1996; Nelson et al., 2003; Engel et al., 2006; Puglisi et al., 2007). Rasher and colleagues have demonstrated that while both whole algal thalli and crude algal extracts elicit coral bleaching, decreased photosynthesis, and in some cases mortality, no impacts were observed from plastic algal mimics and the effects were much more severe from crude extracts than whole thalli (Rasher et al., 2010; Rasher and Hay, 2010; Rasher et al., 2011).

Macroalgal secondary metabolites have been shown to have varying degrees of inhibitory effects on coral larval settlement. Water conditioned with naturally occurring reef macroalgae reduced settlement of *Acropora millepora* larvae by up to 30%, even when combined with the strong settlement cue of crustose coralline algae (Birrell et al., 2008). Both whole thalli and crude extracts of the seaweed *Dictyota sp.* reduced the survival and settlement of *Porites astreoides* larvae (Paul et al., 2011).

1.4 Thesis Questions and Hypotheses

The research presented in this thesis aims to expand our understanding of the naturally-occurring coral-algal interactions that affect shallow coral reefs around the island of O'ahu, Hawai'i.

Hawaiian coral reefs are not thought to be in danger of full-scale phase shifts to alternate algae-dominated states (Roff and Mumby, 2012; Mumby et al., 2015), yet the history of algal invasions and blooms on Hawaiian reefs demonstrates that these areas are still vulnerable to the effects of increased macroalgal growth.

Naturally-occurring coral-algal interactions are rarely surveyed, which may put us at a disadvantage when considering whether these interactions are increasing over time or if reef areas are becoming more affected by algae growth. The first chapter of this thesis represents the first survey of nearshore O'ahu

reefs seeking to quantify these coral-algal interactions and as such can serve as a valuable baseline and springboard for future investigations.

The second chapter investigates specific effects of invasive *Gracilaria salicornia* mats on the health of *Porites lobata* coral. Such mats can persist on the reef for months or longer (personal observation), where they come into direct contact with coral for a much longer time than is common for more ephemeral species of reef macroalgae. The vast majority of coral-algal interaction experiments have a short duration, most less than 20 days. Because of this, the second chapter reports an experimental exposure lasting 3 months, which compares physiological metrics of the coral over time to better understand the effect of these invasive algal mats on coral at time scales that better reflect the interaction durations observed on Hawaiian reefs.

The research presented in this thesis is focused on the following questions and hypotheses:

Chapter 1. In situ *coral-algal dynamics on shallow reefs around O'ahu, Hawai'i.*
What patterns exist in naturally-occurring coral-algal interactions on reefs around O'ahu?

Hypothesis 1.1 The number of interactions per coral colony differs by location.

Hypothesis 1.2 The severity of algal interactions experienced by coral differs by location.

Hypothesis 1.3 The number of interactions per colony differs by coral species.

Hypothesis 1.4 The severity of interactions experienced by coral differs by coral species.

Chapter 2. Effects of long-term contact with the invasive alga Gracilaria salicornia *on the pan-Pacific coral,* Porites lobata.
Do corals in contact with *G. salicornia* display signals of physical stress? If so, does that response vary over exposure duration?

Hypothesis 2.1 *P. lobata* in contact with *G. salicornia* mats will display altered physiological metrics (coloration, growth rate, zooxanthellae density, pigment composition, and/or lipid content).

Hypothesis 2.2 Physiological stress metrics will differ over contact duration.

Chapter 2

In situ Coral-Algal Dynamics on Shallow Reefs around O'ahu, Hawai'i

2.1 Abstract

Benthic macroalgae are an important source of primary production in coral reef ecosystems, but overfishing and nutrification cause algal growth to outpace herbivore grazing, resulting in a higher than normal biomass of frondose algae competing with corals for space and light on the reef. Naturally-occurring interactions between coral and algae are rarely surveyed, which could put us at a disadvantage when attempting to monitor coral reef health and vulnerability. As the first survey of naturally-occurring coral-algal interactions around the island of O'ahu, Hawai'i, a total of 280m^2 of nearshore reefs were surveyed, encompassing a total of 1293 coral colonies and 240 direct coral-algal interactions. The number of interactions per colony differed significantly between sites and coral species, but averaged 2.2 interactions per m^2 overall. The severity of interactions differed significantly between sites, coral species, and algae species, though the majority of interactions were characterized by normal coloration and tissue. Only 3% of interactions showed coral bleaching, and less than 6% showed signs of tissue abnormality or mortality.

2.2 Introduction

Benthic algae is an important source of primary production in coral reef ecosystems, supporting a large biomass of herbivorous fish and invertebrate species directly as food and habitat. These algae are an essential component of a healthy coral reef, but stresses on the system such as nutrification and overfishing can allow algal growth to outpace herbivore grazing, leading to an overabundance of algae on impacted reefs. Declines in coral cover worldwide (Gardner et al., 2003; Cote et al., 2005; Sweatman et al., 2011; De'Ath et al., 2012) are accompanied by reports of increased fleshy macroalgae on reefs (Bellwood et al., 2004; Cote et al., 2005; Hughes et al., 2007; Chadwick and Morrow, 2011; Mumby and Steneck, 2011). In some regions these changes can lead to a phase shift to an alternate, stable macroalgae-dominated system (Done, 1992; Hughes et al., 2007; Lesser et al., 2007; Ledlie et al., 2007; Maliao et al., 2008). While some studies suggest that the prevalence of full-scale phase shifts globally may not be as high as previously thought

(Bruno et al., 2009) and some ocean basins may have higher intrinsic resilience to alternate stable states (Roff and Mumby, 2012; Mumby et al., 2015), the ecological, economic, and social costs of such events (McManus et al., 2000; Hughes, 2003; McManus and Polsenberg, 2004) are great enough to warrant concern.

Whether or not a reef system is at risk for a complete phase shift, increased prevalence of macroalgae will necessarily increase the prevalence of competitive interactions between coral and algae, both of which require substrate space. Many experimental exposures to and removals of algae have demonstrated that direct interactions between coral and algae are often characterized by a variety of negative competitive mechanisms including abrasion, shading, and smothering (McCook et al., 2001; Hauri et al., 2010; Martinez, 2012), alteration of the immediate physical and chemical environment (Stamski and Field, 2006; Martinez et al., 2012), disease transmission (Nugues et al., 2004; Sweet et al., 2013), disruption of the coral-associated microbial community (Smith et al., 2006; Morrow et al., 2011, 2012b,a; Barott and Rohwer, 2012), allelopathy (Rasher and Hay, 2010; Rasher et al., 2010, 2011), and inhibition of coral larval settlement and metamorphosis (Birrell et al., 2008; Paul et al., 2011). These interactions result in decreased coral fecundity (Foster et al., 2008), growth (Tanner, 1995; Titlyanov et al., 2007), and wound healing (Titlyanov et al., 2005), as well as bleaching and outright mortality (Jompa and McCook, 2003; Rasher et al., 2011).

The extent of naturally-occurring competitive interactions between coral and algae on reefs, as well as the impact of such interactions on coral colonies has not been extensively studied (Haas et al., 2009; Bonaldo and Hay, 2014; Bruno et al., 2014). In fact naturally-occurring rates and severities of interactions have never been quantified on Hawaiian reefs. This study seeks to describe natural patterns of direct interactions between coral and macroalgae on shallow reefs around O‘ahu, Hawai‘i, and the severity of those interactions in terms of coral visual health. This survey will serve as the first description of naturally-occurring coral-algae interactions around the island and as such can be used as a baseline reference for future work.

2.3 Methods

2.3.1 Site Selection

The island of O‘ahu in Hawai‘i, USA, situated at 21’30"N and 157’55"W, is the most populated and developed of the Hawaiian Islands. As such, coral reefs around the island are heavily impacted by sedimentation and pollution from storm water runoff as well as overfishing, dredging, trampling, and invasive species of both fish and algae (Hunter and Evans, 1995; Conklin and Smith, 2005; Birkeland, 2015; Rodgers et al., 2010).

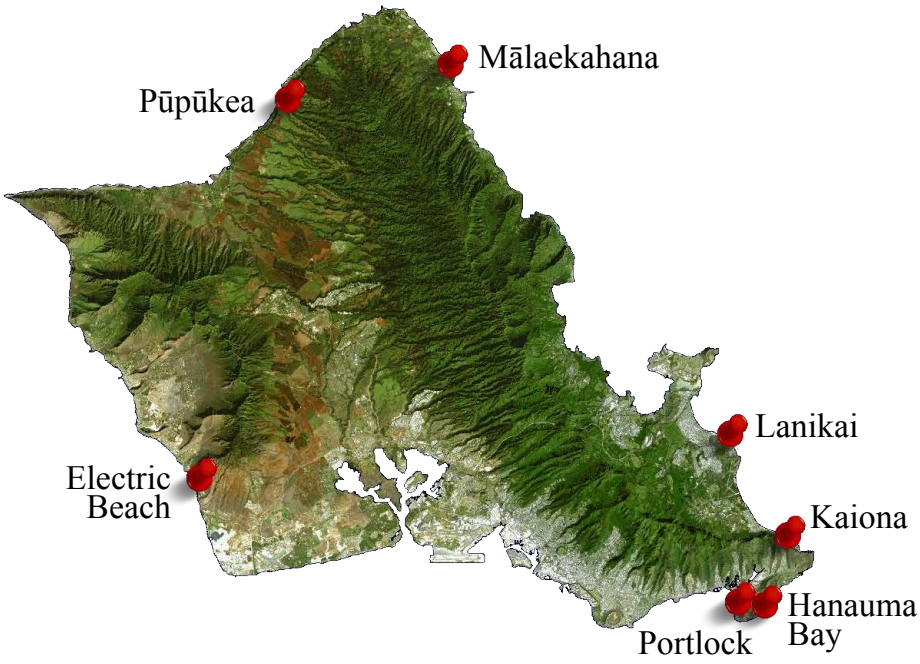

FIGURE 2.1: Location of field survey sites around the Island of O'ahu, Hawai'i.

To investigate the degree of exposure to macroalgae that corals experience on shallow reefs around O'ahu, seven sites were selected for field surveys. These sites, seen in Figure 2.1, were selected in order to encompass a range of environments, exposures, and stressors around the island.

Two of the sites selected are Marine Life Conservation Districts (MLCDs) - protected by state law from fishing or collection of any kind. Pūpūkea on the north shore was established in 1983 and Hanauma Bay on the eastern shore was established in 1967. All sites are popular recreational sites for swimming, snorkeling, diving, and other watersports.

2.3.2 Coral-algae Interaction Surveys

All surveys were completed between June and August 2015. At each of the seven sites, two 10x2m belt transects were run perpendicular to shore in 3-8m depth, for a total of 40m^2 surveyed per site. Every coral colony larger than 3cm that fell within the belt transect was measured, recorded, and checked for interactions with macroalgae. Throughout this paper, "interactions" refers to direct contact between coral and algae tissue (see examples in Figure 2.2).

For every coral-algal interaction, the species of algae, size of the interaction area, and appearance of the coral tissue adjacent to the algae were recorded. See Table 2.2 for an explanation of the coral tissue appearance scores that were used. Photos in Figure 2.2 demonstrate examples of

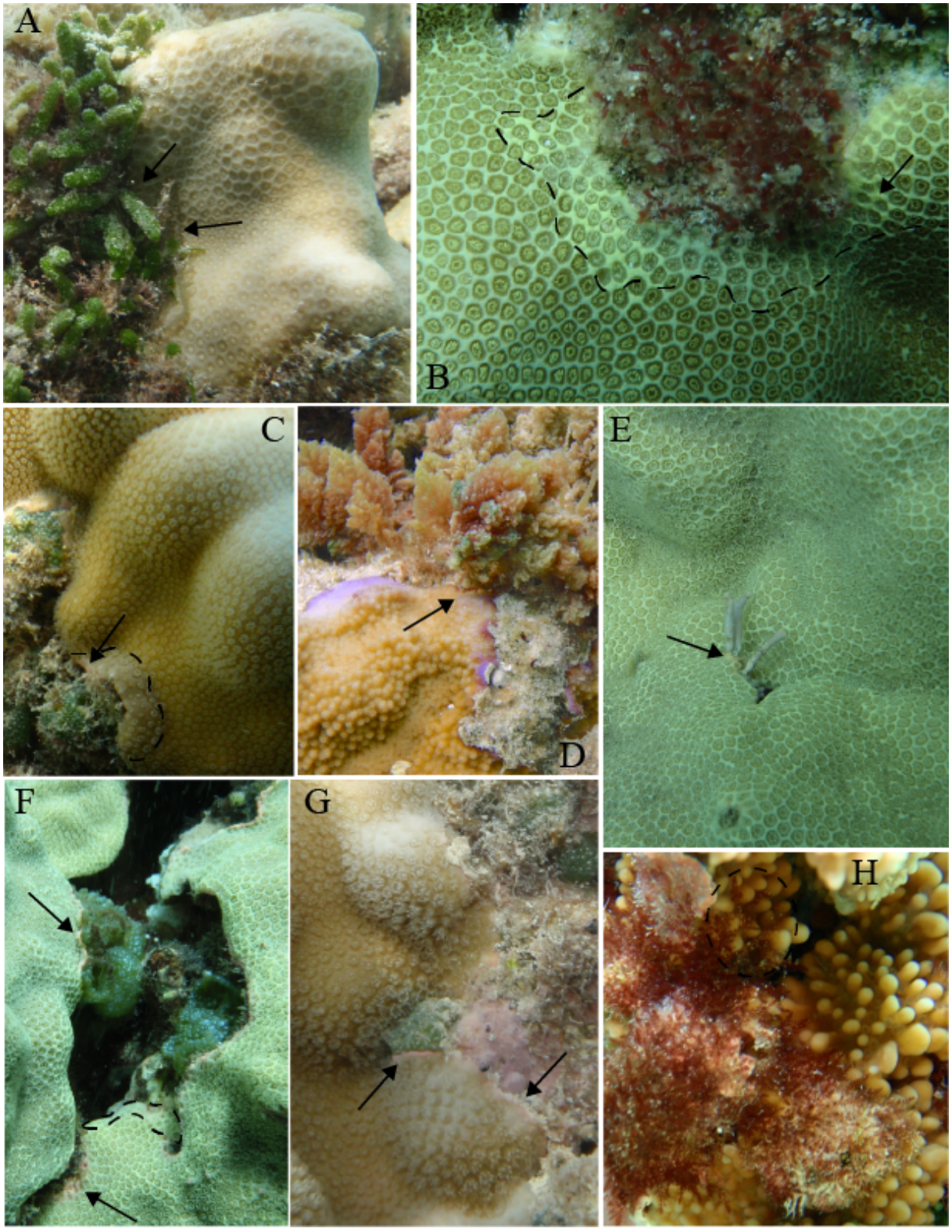

FIGURE 2.2: Examples of coral-algae interactions and interaction visual appearances observed on field surveys. "Interactions" here and throughout refers to direct physical contact between coral and algae tissue, such as is seen in the above photos.

TABLE 2.1: Coral Appearance Scores. Coral tissue directly in contact with algae at each interaction zone was given one score for color (0,2-4) and one score for tissue integrity (0-4). Total severity scores used in a later analysis are the sum of color score + tissue score for a range of severity from 0 to 8. See Figure 2.2 for examples.

Score	Appearance
	Coloration
0	Normal Color - Matching the rest of the colony (not bleached)
2	Discolored - Pinkish hue or other non-normal coloration
3	Pale - Lighter color at interaction zone
4	Bleached - Bleached tissue at interaction zone
	Tissue Integrity
0	Normal Tissue - Matching the rest of the colony
1	Interaction Boundary - "no man's land" area of demarcation between coral and algal tissue
2	Direct Overgrowth - algae tissue growing directly on top of living coral tissue
3	Tissue Abnormality - puffy or otherwise abnormal appearance
4	Tissue Loss - partial tissue mortality at interaction zone

these scores: 2.2A) Normal tissue color and appearance in interaction zone (color=0,tissue=0, table 2.1); 2.2B) Zone of obvious pale coloration with abnormal, puffy tissue appearance at arrowhead (color=3,tissue=3); 2.2C) Zone of abnormal tissue appearance with pink discoloration at arrowhead (color=2,tissue=3); 2.2D) Coral tissue next to algae looks no different from coral margins not adjacent to algae, and is considered normal despite the purple coloration (color=0,tissue=0); 2.2E) Pink discoloration observed at top arrowhead (color=2,tissue=0); 2.2F) Multiple zones of pink discoloration and tissue abnormality within dashed lines (color=2,tissue=3); 2.2G) Pink discoloration at left arrowhead (color=2), right arrowhead marks a small interaction boundary (tissue=1); 2.2H) Direct overgrowth observed within dashed circle (color=0,tissue=2).

All species identifications are field identifications and limited to visual macroscopic characteristics. Corals of the genus *Porites* that were not obviously distinguishable as *P. compressa* were called *P."lobata"*, since distinguishing between *P. lobata*, *P. lutea*, and *P. evermanni* in the field is challenging. Similarly, *Pocillopora* corals have a wide range of morphologies and recent evidence (Johnston et al., 2017) suggests that conclusive identification is dependent on genetic analysis. In this paper, *P. damicornis* represents the *P. "damicornis" / P. acuta* species complex. Algae were identified to genus level except in the case of species with macroscopic distinguishing characteristics or for those genera that are only represented by one species in Hawai'i.

2.3.3 Data Analysis

The number of distinct interactions (defined as one continuous area of direct contact with an individual alga) experienced by each coral colony was analyzed for differences in mean variance between coral species and sites. Because the data were zero-inflated, but more dispersed than

a standard Poisson distribution, a quasipoisson method was used, which uses the dispersion parameter to correct the confidence and p-values returned by an F-test.

The visual health of each individual interaction zone was ranked according to coloration and tissue appearance, and these two scores were summed to create a total severity index score. All three parameters were analyzed for differences in mean variance between coral species and site as well as algae species and site, using the quasipoisson method to account for an overdispersed poisson distribution.

2.3.4 Environmental Variables

A total of 23 environmental predictor variables were considered for inclusion in the models (Table 2.2). These variables consisted of oceanographic stressors (i.e., fishing pressure, population proximity, runoff quantity and type, shoreline development) that may undermine the stability of nearshore reef systems. These data were sourced from layers created by Lecky (2016) and Wedding et al. (2018). Each predictor layer was georeferenced and matched to corresponding site data.

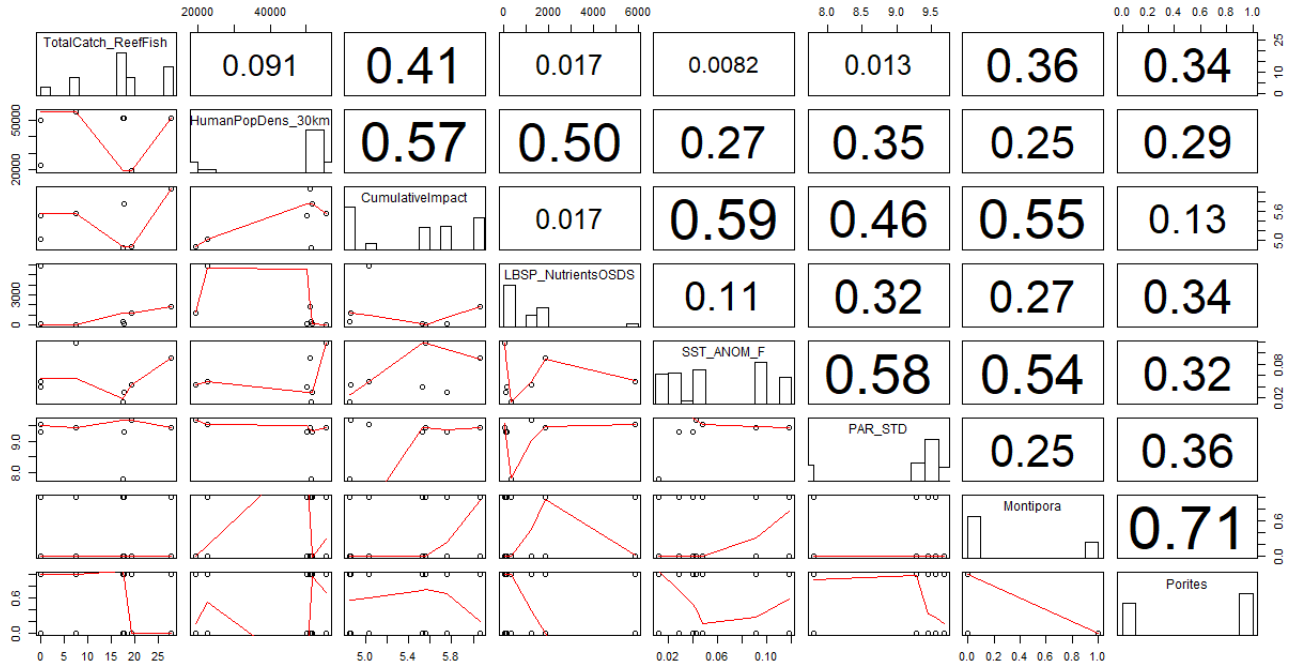

FIGURE 2.3: Correlation scatterplots and values for the final variables included in the models.

Fourteen variables were excluded from the initial model due to high (0.7 or greater) correlation with other predictors (Tabachnick and Fidell, 1994; Dancey and Reidy, 2004). The final

TABLE 2.2: Environmental predictor variables considered for use in the interaction severity model. Data sourced from Wedding et al. (2018) and Lecky (2016)

Variable	Units	Description
TotalCatch Reef Fish	kg/ha/yr	Estimated total average annual catch of reef fish, 2004-2013. All gears, platforms, and sectors combined.
AqCatch	#/ha/yr	Average annual reported commercial aquarium catch (2003-2015).
PopDens 15km	# people	Human population living within 15km, based on dasymetrically mapped 2010 census data.
PopDens 30km	# people	Human population living within 30km, based on dasymetrically mapped 2010 census data.
CumImpact	NA (index)	Cumulative impact score based on 20 stressor maps and expert knowledge derived habitat vulnerability scores (Lecky, 2016).
CI NoClim	NA (index)	Cumulative impact score excluding all climate change layers.
LBSP Sediment	tons/yr/ha	Estimated average annual sediment load, modeled with a custom INVEST sediment delivery model and simple offshore plume function.
LBSP Nutrients	g/day/km^2	Flux of Nitrogen from on-site sewage disposal systems (cesspools, septic tanks) entering the groundwater within a 1.5km radius.
LBSP AgGolf	NA (proxy)	Runoff based on agriculture land (2010) and golf courses (2016) in USGS HUC12 watersheds. Built-in Gaussian decay function.
LBSP Urban	NA (proxy)	Developed impervious surface in USGS HUC12 watersheds from NOAA CCAP 2010/2011. Built-in Gaussian decay function.
LBSP CI	NA (index)	Cumulative impact score for land-based pollution stressors.
Development	NA (proxy)	Change from an undeveloped class to impervious surface 2005-2010.
SST CLIM_M	degrees C	Sea Surface Temperature from AVHRR sensor 2000 - 2013; Maximum Monthly Climatological Mean.
SST ANOM_F	#anomalies /yr	Sea Surface Temperature from AVHRR Sensor 2000 - 2013; Average Annual Frequency of Anomalies.
SST STC	degrees C	Sea Surface Temperature from AVHRR sensor 2000 - 2013; Standard Deviation of Long-term Mean.
WAV LTM	kW/m	Wave power derived from the UH SWAN model (Simulating Waves Nearshore), 2000 - 2013; Long-term Mean.
WAV ANOM_M	kW/m	Wave power derived from the UH SWAN model (Simulating Waves Nearshore), 2000 - 2013; Average Annual Maximum Anomaly.
WAV ANOM_F	#anomalies /yr	Wave power derived from the UH SWAN model (Simulating Waves Nearshore), 2000 - 2013; Average Annual Frequency of Anomalies.
CHL CLIM_M	mg/m^3	Chlorophyll-a concentration from NASA MODIS Aqua, 2003 - 2013; Maximum Monthly Climatological Mean.
CHL LTM	mg/m^3	Chlorophyll-a concentration from NASA MODIS Aqua, 2003 - 2013; Long-term Mean.
CHL ANOM_F	#anomalies /yr	Chlorophyll-a concentration from NASA MODIS Aqua, 2003 - 2013; Average Annual Frequency of Anomalies.
PAR LTM	Einstein /m^2/day	Photosynthetically active radiation (irradiance) from NASA MODIS Aqua, 2003 - 2013; Long-term Mean.
PAR STD	Einstein /m^2/day	Photosynthetically active radiation (irradiance) from NASA MODIS Aqua, 2003 - 2013; Standard Deviation of Long-term Mean.

model included the following predictors: mean annual reef fish biomass catch, human population living within 30 kilometers of the site, cumulative habitat vulnerability, nitrogen flux from sewer systems, mean annual frequency of sea surface temperature anomalies, and standard deviation of irradiance (Figure 2.3). See Table 2.2 for complete covariate metadata.

2.3.5 Model Construction

Sufficient data was collected to build models for corals of genus *Porites* (n=165) and *Montipora* (n=91). To model the environmental drivers influencing the severity of interactions, we constructed hierarchical models for each coral genus, denoted as

$$\mu_{\mathrm{i}} = \beta_0 + \alpha_1 X_{1\mathrm{i}} + ... + \alpha_{\mathrm{k}} X_{\mathrm{ki}} + \epsilon$$

Here, μ_{i} is the expected value for observation i; the coefficients corresponding to predictors X_{i} at sites $k(k = 1, 2, 3...6)$ are given by α_{i}. We included site specification as a random effect to account for differences in mean severity at different sites. We assumed that each site mean ϕ_{k} is normally distributed $(\phi_{\mathrm{k}} \sim N(\mu_\phi, \sigma_\phi))$. Each observation y is also assumed to be distributed normally $(y_{\mathrm{i}} \sim N(\phi_{\mathrm{ij}}, \sigma_{\mathrm{y}})$. Best-fit models were selected based on AICc values, standard error estimates for the covariates, and examination of the response variable residuals using quantile-quantile plots.

R statistical software (Team, 2016) was used to perform all data analyses. Models were specified using the "lme4" package. The Kenward-Roger approach (Schaalie et al., 2002) and the R package "r2glmm" (Jaeger, 2017) were used to calculate R^2 values for the models and partial R^2 values for the fixed effects.

2.4 Results

For each of seven sites around the island of O'ahu, 40m^2 were surveyed for a total of 280m^2 surveyed reef area. Overall, an average 184 coral colonies were surveyed per site, 158 colonies per species.

The most commonly occurring coral species on survey transects (Figure 2.4b) were *Porites lobata* (47%), *Montipora capitata* (16%), *Montipora patula* (14%), *Pocillopora meandrina* (10%), and *Pocillopora damicornis* (5%). The most commonly observed macroalgae species observed in interactions with coral (Figure 2.4a) were *Lyngbya majuscula* (Cyanophyceae, 31%), *Dictyosphaeria versluysii* (Chlorophyta, 20%), *Halimeda spp.* (Chlorophyta, 17%), *Asparagopsis taxiformis* (Rhodophyta, 10%), and *Laurencia spp.* ("limu lipe'epe'e," Rhodophyta, 6%). Given those relative frequencies, the most commonly observed coral-algal interaction pairs are not surprising. The five most common pairs (Figure 2.4c) were *P. lobata & D. versluysii* (14%), *P. lobata & Cyanobacteria spp.* (10%),

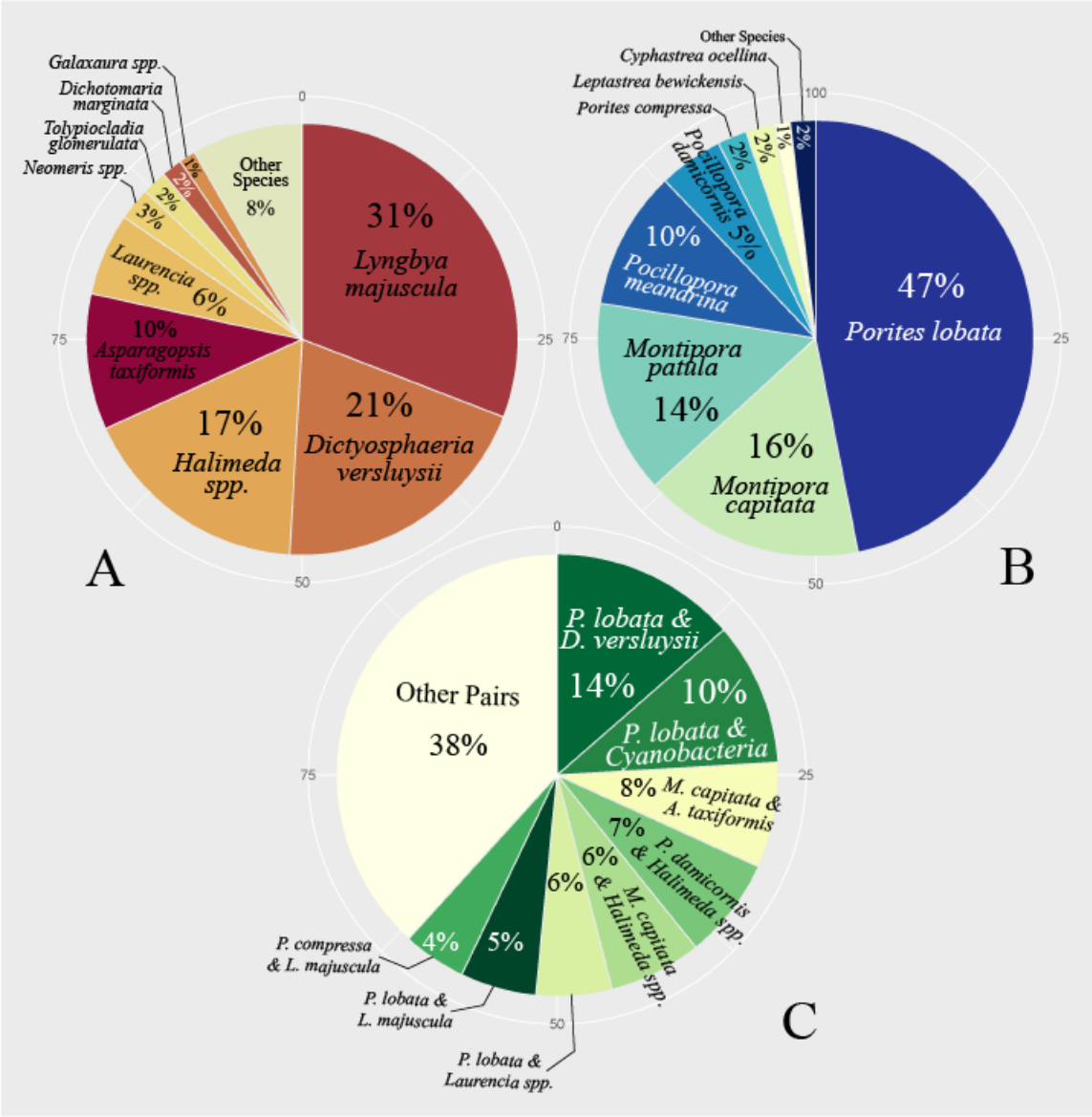

FIGURE 2.4: Relative frequencies of species observed. A) Algae species observed in interactions with coral. Other species were those that made up less than 1% of observations each. B) Coral species observed over entire transect area. Other species were those that made up less than 1% of observations each. C) Most frequently observed coral-algae interaction pairs. Other pairs were those that made up less than 3% of observations each.

M. capitata & A. taxiformis (8%), *P. damicornis & Halimeda spp.* (7%), and *M. capitata & Halimeda spp.* (7%).

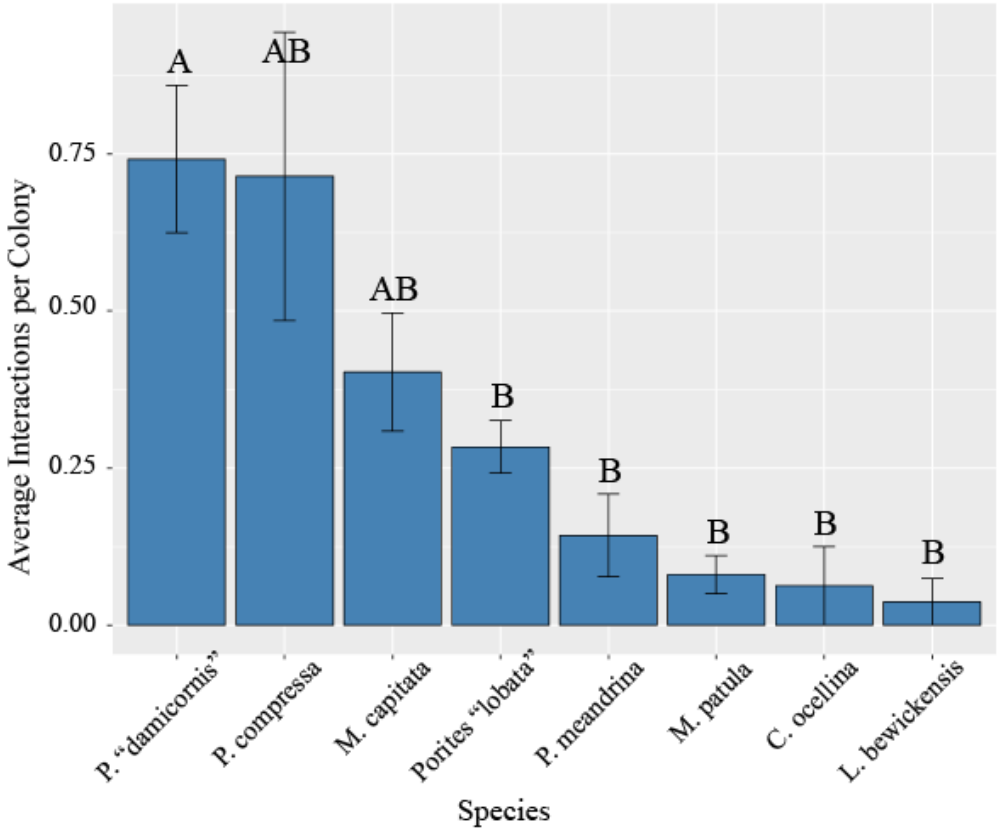

FIGURE 2.5: Average number of interactions per coral colony by species (shown are only those coral species observed in more than 10 interactions) with standard error bars, letters denote Tukey's post-hoc test groups.

2.4.1 Number of Interactions by Species and Location

The average number of direct contact interactions with macroalgae per coral colony was calculated to be 0.28 overall. Of the 1293 colonies recorded within the transect areas, 53% did not have any direct interaction with macroalgae, while the maximum number of interactions recorded for a single coral colony was 18.

The F-test of the full linear model of the number of interactions by coral species and site returned p-values of 0.66 ($F_{15/1271}$ = 0.82) and <0.001 ($F_{6/1271}$ = 11.8), respectively. Analysis of the interaction between coral species and site was not possible because not all species are represented at all sites. When we removed the independent variable of Site and just examined the number of interactions by coral species, there were significant differences (p = 0.001, $F_{15/1277}$ = 2.53, Figure 2.5). The species experiencing the highest number of interactions with macroalgae was *P. damicornis*, with an average of 0.74 interactions per colony. The highest number of interactions per colony was observed at Kaiona (0.95, Windward coast), while the fewest interactions

per colony were observed at Pupukea (0.08, North Shore) and Hanauma Bay (0.03, East Side, Figure 2.6).

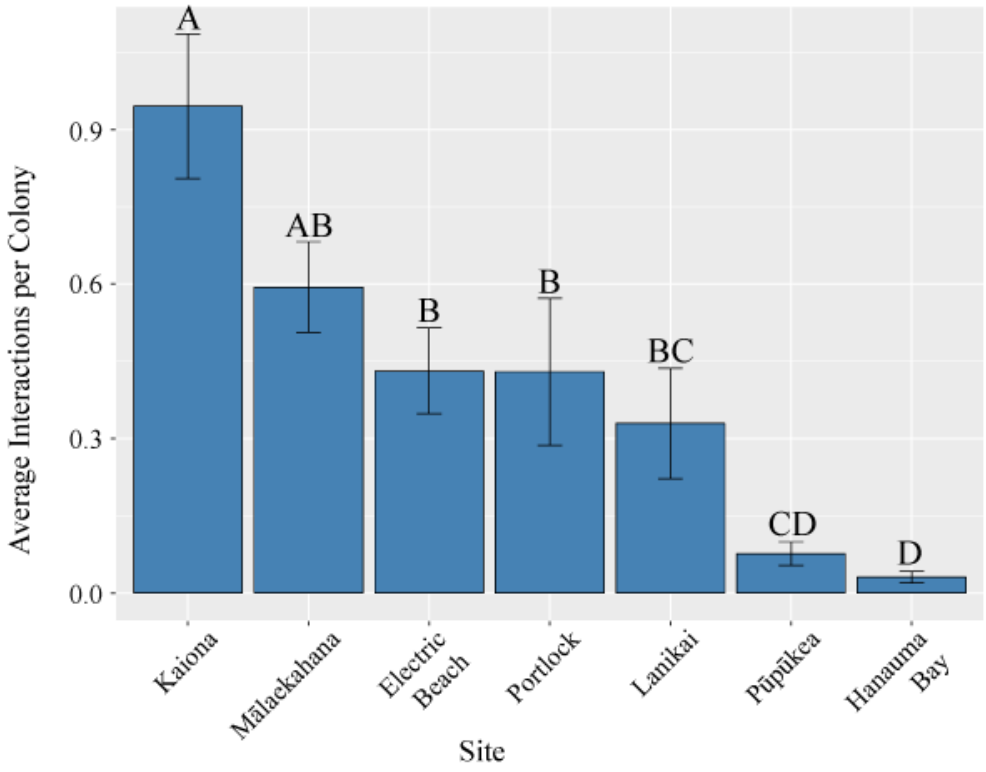

FIGURE 2.6: Average number of interactions per coral colony by site with standard error bars, letters denote Tukey's post-hoc test groups.

2.4.2 Severity of Interactions by Species and Location

A total of 240 direct interactions were observed between macroalgae and coral tissue. For each of these instances, the coral tissue in the interaction zone was given two scores: one for color and one for tissue (see Table 2.1). For the purpose of overall comparisons, the two scores for each interaction were added together to create a total severity score with a range of 0-8. The maximum severity score for observed interactions was 7, with an overall average of 1.8.

Overall, 33% of interactions were characterized by normal color and tissue appearance. 19% of all coral tissue in an algae interaction was discolored, 14% was pale, and only 3% was bleached. Similarly, 16% of interactions displayed direct overgrowth of algae tissue onto coral tissue, and only 6% showed any signs of tissue abnormality or mortality.

The visual health score for coloration at interaction zones differed significantly between coral species ($F_{9/298} = 3.48$, $p = 0.0004$) and sites ($F_{6/298} = 9.58$, $p < 0.001$), with the interaction between species and site also significant ($F_{10/298} = 2.62$, $p = 0.004$). *Montipora patula* displayed the least

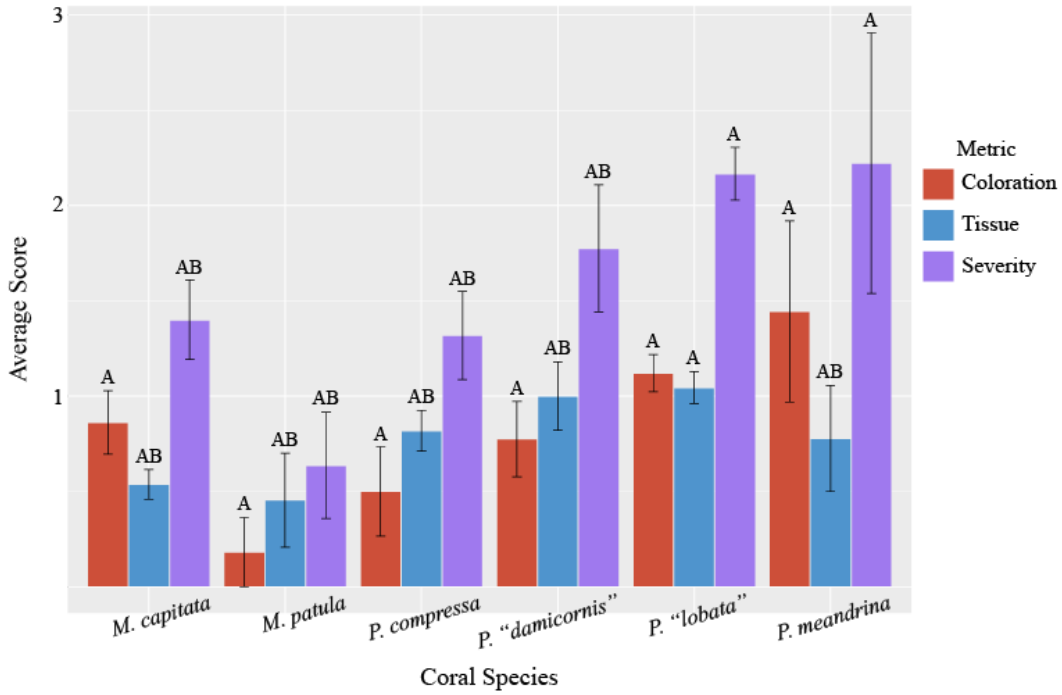

FIGURE 2.7: Average visual health (color, tissue, and combined severity) scores by coral species with standard error bars, letters denote Tukey's post-hoc test groups.

alteration to coloration in interactions, while *Pocillopora meandrina* displayed the most (average color score 0.18, 1.44, respectively; Figure 2.7). The site where interactions displayed the most altered coloration was Electric Beach (South Shore, 1.47), while the interactions at Kaiona (Windward, 0.26) were the least abnormally colored .

In the full model of tissue integrity by coral species and site, tissue integrity scores were found to differ significantly by site ($F_{6/308}$ = 2.80, p = 0.01), but not by coral species ($F_{9/308}$ = 0.69, p = 0.72). Electric Beach (South Shore) had interactions with the highest degree of tissue disruption (1.29), while Pupukea (North Shore) had interactions with the least degree of tissue disruption (0.44, Figure 2.8). However, when the model is reduced to include just coral species without the effect of site, scores are seen to differ significantly by coral species (p = 0.01, $F_{9/314}$ = 2.56). *Montipora capitata* and *M. patula* experienced the least tissue disruption in interactions with algae (avg scores = 0.54, 0.45 respectively) while *P. lobata* experienced the most (avg score = 1.04).

As a combined score, severity did differ significantly between both coral species ($F_{9/298}$ = 2.22, p = 0.02; Figure 2.7) and site ($F_{6/298}$ = 9.33, p <0.001), with the interaction again being significant ($F_{10/298}$ = 3.29, p = 0.0005). The overall severity index (sum of color and tissue scores) was significantly higher for both *P. meandrina* and *P. lobata* than other species (2.22, 2.17, respectively).

Similarly, the severity indices differ significantly between algae species (Color $F_{24/293}$ = 2.22,

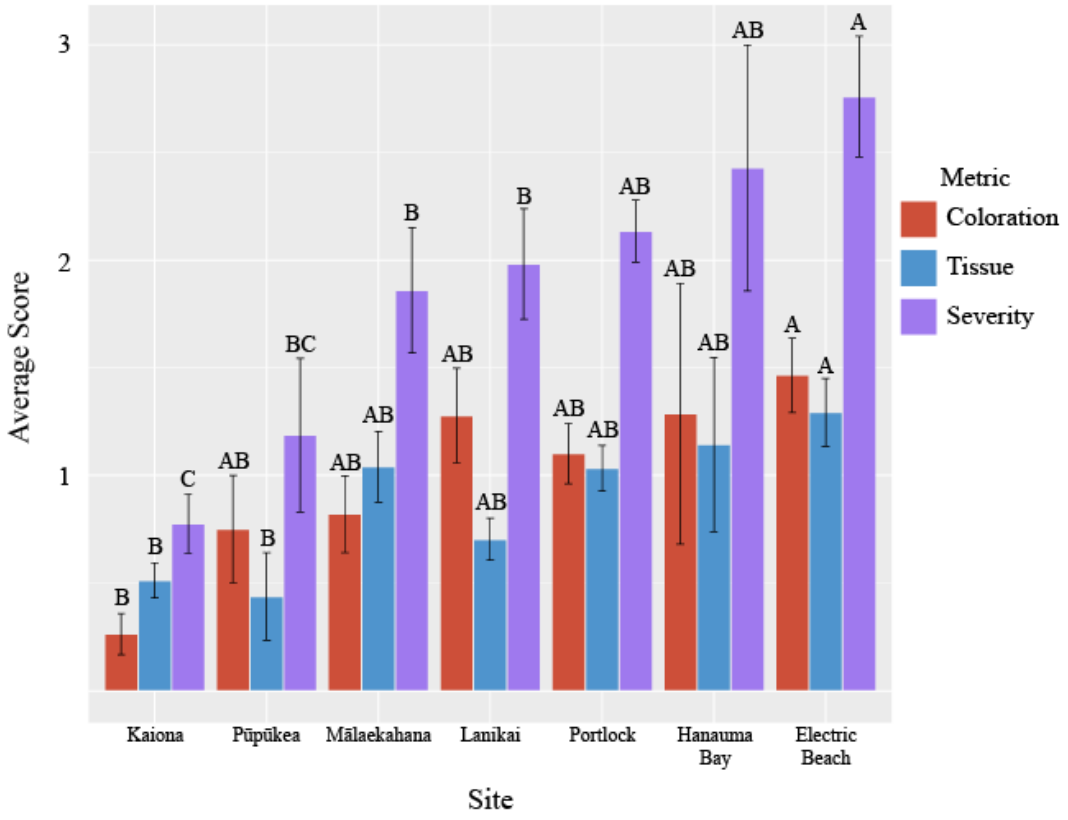

FIGURE 2.8: Average visual health (color, tissue, and combined severity) scores by site with standard error bars, letters denote Tukey's post-hoc test groups.

p = 0.001; Tissue p <0.001, $\chi 2 = 72.67$, Df = 24; Severity $F_{24/293} = 3.44$, p <0.001; Figure 2.9) and site (Color $F_{6/293} = 4.76$, p = 0.001; Tissue p = 0.04, $\chi 2 = 13.25$; Severity $F_{6/293} = 4.59$, p <0.001; Figure 2.9). The algae species that impacted coral the most severely in direct interactions was *Laurencia spp.* (color = 1.75, tissue = 1.40, severity = 3.15), while *Asparagopsis taxiformis* was consistently associated with the least severe coral impacts (Color = 0.18, Tissue = 0.06, Severity = 0.24).

2.4.3 Environmental Drivers

The final models for both *Porites* and *Montipora* converged and showed no evidence of troublesome patterns in model residuals. For both models, frequency of sea surface temperature anomalies accounted for the majority of the variance explained by the model (*Montipora*: R^2= 0.19, partial SST anomaly R^2=0.18; *Porites*: R^2=0.97, partial SST anomaly R^2=0.96). For the *Montipora* model the confidence intervals for all fixed variable estimates were very wide, restricting our ability to make inferences about the influence of any of the included environmental predictors on the variation observed in interaction severity for *Montipora* corals. The confidence

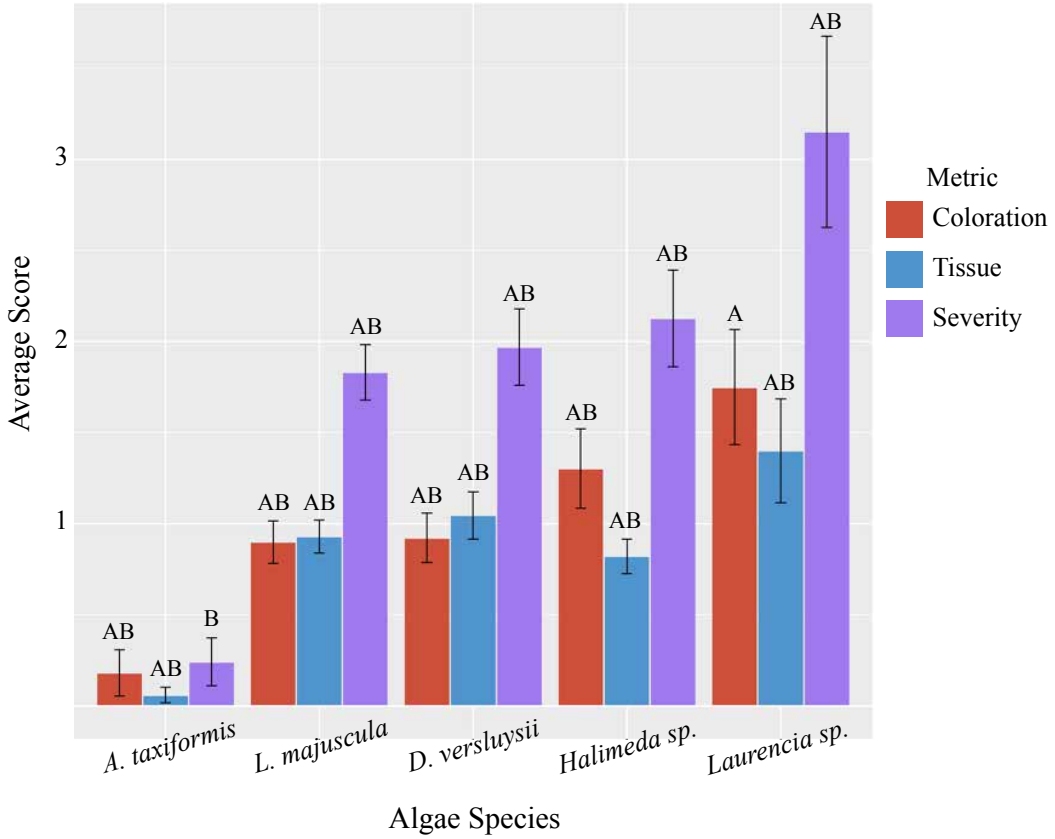

FIGURE 2.9: Average visual health (color, tissue, and combined severity) scores by algae species with standard error bars, letters denote Tukey's post-hoc test groups.

intervals for the *Porites* model on the other hand were fairly narrow, allowing more confidence in the influence of SST anomalies on the interaction severity experienced by *Porites* corals. The standard deviation of irradiance was also found to minimally influence *Montipora* interaction severity. A parametric bootstrap was implemented to determine the significance of the random effect (Site) in each model, which was found to be significant ($p < 0.05$) for the *Montipora* model, but not significant for the *Porites* model, meaning that variation in severity of *Porites* interactions is not explained on a site-by-site level in this model.

2.5 Discussion

Direct coral-algal interactions around the island of O'ahu, Hawai'i appear to be highly affected by coral species, algae species, and site. The observed rate of interaction overall was 0.28 interactions per colony. 47% of the 1293 colonies surveyed over a total of 280m^2 experienced at least

one direct interaction with a macroalga, giving an overall interaction density of about 2.2 interactions per m^2. This is comparable to that found by Haas et al. (2009) in the Gulf of Aqaba (Red Sea), who reported a range of $2.1/m^2$ in the summer to $3.8/m^2$ in the winter. High latitude reefs such as those in the Red Sea experience a high seasonal fluctuation of algal abundance (Bruno et al., 2014), and season was found to significantly structure the coral-algal interactions observed in the Red Sea over a 19-month interval, though monitored levels of nutrients and temperature were not observed to correlate with fluctuations in coral-algal interaction intensity (Haas et al., 2009). This observed coral-algal interaction rate is much lower than that reported for Heron Island, Australia, where 92% of corals were observed to experience at least one distinct interaction with an alga (Tanner, 1995), though our study did not include encrusting crustose coralline algae or turf assemblages.

2.5.1 Site Effects

Strong variations were observed in this study between sites in both the number and severity of direct algal interactions experienced by reef corals around O'ahu. Those sites observed with the fewest coral-algal interactions were Pūpūkea and Hanauma Bay (Figure 2.6), the only included sites that are Marine Life Conservation Areas (MPA's) and are protected from all collection activities. The protection of and resulting theoretical higher biomass of herbivore species keeps coral reef macroalgae at a lower standing biomass through top-down control. Bonaldo and Hay (2014) found a similar result in Fiji, where non-MPA sites had 70-80% fewer herbivores, 4-9 times more macroalgae, and 51-68% less coral cover, resulting in 5-15 times more frequent and 23-67 times more extensive coral-algal interactions (Bonaldo and Hay, 2014). While this result is expected, this set of surveys does not provide enough power to test whether this effect is attributable to protection status and applicable to other regions.

The severity of coral-algal interactions followed a different pattern (Figure 2.8), with interactions in Hanauma Bay observed to be much more severe than those experienced anywhere other than Electric Beach. Kaiona, an unprotected, heavily used recreational site (and the one observed with the highest rate of interactions) was observed with the least severe coral-algal interactions. Damage from coral-algal interactions was found to be independent of study site in Fiji (Bonaldo and Hay, 2014), while coral-algal interaction surveys in the Line Islands of the Pacific found that corals were better able to compete against encroaching algae in sites around uninhabited islands than inhabited (Barott et al., 2012).

The environmental parameters hypothesized to contribute to the observed differences in interaction severity between O'ahu sites were investigated through model fitting. Because of the nature of field surveys, most species were not represented by a high enough sample number to include, so only *Montipora spp.* and *Porites spp.* were selected and separate models were

run with each. We found that average annual frequency of SST anomalies and overall habitat vulnerability influenced the severity of *Montipora* and *Porites* interactions with various species of algae, though the coefficient estimates for all fixed effects displayed wide confidence intervals, indicating that more sampling is needed to draw firm conclusions about the influences of these variables on coral and algae interactions. These analyses suggest that more deleterious algae interactions will occur for *Montipora* colonies at sites with more severe cumulative anthropogenic impacts, while *Porites* colonies will experience more deleterious effects in regions with more severe cumulative anthropogenic impacts when also exposed to higher frequencies of SST anomalies.

2.5.2 Species Effects

When site was removed as a factor, coral species was also highly significant in describing the variation in interaction number and severity on sites around O'ahu. *Pocillopora damicornis*, a low-growing, delicately branched, weedy coral (Darling et al., 2012) experienced the highest rate of direct interactions of all observed species (Figure 2.5). *Montipora capitata*, an encrusting, plating, or crudely branched coral with a fast-growing, competitive life history strategy (Darling et al., 2012), experienced an intermediate rate of interactions. *Porites lobata*, a large mounding or occasionally encrusting coral with a stress-tolerant life history strategy (Darling et al., 2012), grouped statistically with those corals experiencing the lowest rate of interaction - the majority of which were not observed in any direct interactions with algae. The impact of the observed interactions on the visual health of the coral was most severe for *Pocillopora meandrina* (a small, but robust branching coral) and *P. lobata*, with *M. patula* (a fast growing, encrusting species) experiencing the least severe interactions overall.

Life history strategy and colony morphology have been implicated in affecting the outcome of coral-algal interactions (Hughes, 1989; Tanner, 1995; Lirman, 2001; Nugues and Bak, 2006; Haas et al., 2009), though the observed patterns are sometimes conflicting. In Florida, encrusting *Porites astreoides* was negatively affected by increasing interaction pressure (through caging), while two massive coral species had opposite reactions, with *Siderastrea siderea* showing no effect of increased competition and *Montastrea faveolata* being highly affected (Lirman, 2001). On Heron Island, most species' health was not significantly affected, but those with a more 3-dimensional growth forms (branching or mounding rather than encrusting) encountered fewer algae (Tanner, 1995). In the Red Sea, branching corals were seen to be more often affected by contact with algae than massive colonies (Haas et al., 2009). In Fiji, *P. lobata* was observed to recover quickly from damage, while *P. damicornis* did not (Bonaldo and Hay, 2014). The observed patterns on O'ahu show small, encrusting species (*M. patula*, *Leptastrea bewickensis*) encountering very few algae, and being less severely impacted when they do contact algae. The massive, "stress tolerant" *P.*

lobata encountered few algae comparatively, but was highly affected by those interactions, and the fast-growing, competitive species (*M. capitata*, *P. compressa*) displayed an intermediate rate and severity of interactions. *P. damicornis* was expected to be most highly impacted by algae because of its low, highly branching growth form, thin tissue, and weedy nature, but while it did experience the highest rate of interaction, those interactions were only moderately severe and were significantly less severe than those experienced by the "stress tolerant" *P. lobata*.

Coral reef macroalgae differ substantially in their characteristics, competitive strategies, and impacts on coral (Jompa and McCook, 2003; Fong and Paul, 2011). Cyanobacteria are known to have detrimental effects on coral health (Haas et al., 2009; Titlyanov et al., 2007). The common cyanobacterium *Lyngbya majuscula* was the most prevelant alga in coral-algal interactions on O'ahu, and caused an intermediate amount of visual health alteration to the corals they contacted. *Laurencia sp* was associated with the most severe interactions in this study (though it made up only 6% of interactions), followed by *Halimeda sp* and *Dictyosphaeria versluysii*, which represent the third and second most (respectively) prevalent algae in coral-algal interactions. *L. majuscula*, *D. versluysii*, and *Halimeda sp.* combined account for 70% of the observed interactions on O'ahu during this study. *Laurencia sp.* is chemically defended with antifungal and antibacterial agents (Engel et al., 2006; Gross, 2003) and has previously been seen to kill unsettled coral larvae (Birrell et al., 2008), which may explain the more severe impact of this alga on coral during this study.

2.5.3 Conclusion

As the first survey of *in situ* coral-algae interactions on reefs around O'ahu, Hawai'i, this study can act as a baseline as well as a springboard for further research. Because of the nature of field surveys and the natural differences in coral and algal communities between sites, the patterns and results reported by this study cannot be extrapolated to other locations. In general, however, this study supports previous findings that coral species, morphology and/or life history highly structure coral-algal interactions, that environmental conditions influence the rate and severity of interactions, and that naturally-occurring interactions are less severe than experimentally-manipulated interactions (Tanner, 1995; Lirman, 2001; Haas et al., 2009; Barott et al., 2012; Bonaldo and Hay, 2014) and therefore represent an important gap in our understanding of coral reef ecosystem dynamics.

Chapter 3

Effects of Long-term Contact with the Invasive Alga *Gracilaria salicornia* on *Porites lobata*

3.1 Abstract

Research on coral-algal interactions has increased in recent years in response to reports of increased macroalgal biomass on coral reefs worldwide, attributed to local stressors that favor macroalgal growth while reducing the health, growth, and recruitment of coral. While many studies have demonstrated the ability of macroalgae to have dramatic negative effects on corals they contact, most experiments measure only short-term responses, with exposures lasting less than 20 days.

On O'ahu, Hawai'i, coral reefs are affected by the invasive red alga, *Gracilaria salicornia*, which forms thick, persistent mats around and over coral colonies, resulting in coral-algae interactions surpassing the time frame typically investigated. In order to assess the effect of this invasive algal mat on the primary reef-building coral in Hawai'i, *Porites lobata*, nubbins of the coral were placed in direct lateral contact with invasive algal mats for 1, 2, and 3 months.

In keeping with previous short-term studies, coral nubbins experiencing direct contact with algal mats exhibited higher prevalence and severity of bleaching, reduced photosynthetic pigment concentrations, and altered lipid content. For all physiological metrics used in this study (except growth rate), the 1 month exposure treatment was the most dissimilar to the control group. Several metrics returned to near baseline conditions after 3 months, suggesting that the response of this coral to side-by-side contact with *G. salicornia* mats is more immediate and acute than chronic.

3.2 Introduction

Recent studies report increased proliferation of macroalgae on coral reefs, likely due to a combination of local and global stressors resulting in fewer herbivores left to eat faster growing, less palatable algae. Declines in coral cover have been reported in all ocean basins (Gardner et al., 2003; Cote et al., 2005; Bruno and Selig, 2007; Sweatman et al., 2011; De'Ath et al., 2012), accompanied by an observed increase in macroalgal cover (Bellwood et al., 2004; Cote et al., 2005; Hughes

et al., 2007; Chadwick and Morrow, 2011; Mumby and Steneck, 2011). Any increased biomass of macroalgae on coral reefs leads to an increased prevalence of direct interactions between benthic coral and algae as they compete for space on the substrata. This increase in coral-algal interactions on reefscapes is reflected by the increase of coral-algae interaction research in recent years (Barott and Rohwer, 2012).

This growing body of coral-algae interaction research supports the view that algae directly compete with corals on the benthos through a variety of mechanisms. These include direct physical effects of shading, abrasion, and smothering (McCook et al., 2001; Hauri et al., 2010) as well as altering the immediate physical and chemical environment by decreasing irradiance, decreasing water flow, increasing sedimentation, and increasing daily pH and oxygen fluxes (Stamski and Field, 2006; Martinez et al., 2012).

Many algae are also chemically defended by reactive oxygen species and secondary metabolites (Cronin and Hay, 1996; Engel et al., 2006; Rasher and Hay, 2010; Rasher et al., 2011; Puglisi et al., 2014). A recent study showed that macroalgae were more palatable to herbivores after being in competition with coral, suggesting that some species produce specific anti-coral secondary metabolites that are in a trade-off with anti-herbivore secondary metabolites (Longo and Hay, 2015).

Direct contact between coral and algal tissues also brings into contact distinct microbial communities (Rohwer et al., 2002; Barott et al., 2011; Morrow et al., 2012a,b), which may introduce novel microbes to the coral holobiont community, including known coral pathogens (Nugues et al., 2004; Barott et al., 2011; Sweet et al., 2013). Leached photosynthates and dissolved inorganic compounds from macroalgal thalli also increase coral microbial activity (Steinberg et al., 2002; Kline et al., 2006), which may decrease immune function, increase virulence, and in some cases cause rapid mortality to the coral animal (Smith et al., 2006).

Experimental exposures to macroalgal thalli or extracts have shown that some algae species can have severe and rapid negative effects on coral. Bleaching is a common response of corals experiencing algae exposure stress, sometimes followed by tissue loss, which may lead to partial or full mortality. These effects tend to be heightened by exposing coral to algal extracts compared to whole algal thalli (Rasher et al., 2010, 2011; Rasher and Hay, 2010).

Corals affected by competition with macroalgae not only have reduced growth rates, contributing to the ability of fast-growing algae to outgrow and out-compete them, but also experience reduced wound healing (Titlyanov et al., 2005), which may increase their susceptibility to disease and predation. Many coral diseases are thought to be opportunistic infections that strike colonies experiencing other forms of stress (Lesser et al., 2007), such as competition with algae. Some algae are even known to act as disease reservoirs, actively hosting and transferring virulent pathogens to coral they contact (Nugues et al., 2004; Sweet et al., 2013).

The Hawaiian island of O'ahu has coral reefs characterized by high coral cover, low coral diversity, and high rates of endemism (Kay and Palumbi, 1987; Allen, 2008). These reefs are also affected by several invasive algae species, notably *Gracilaria salicornia* - a red agarophyte alga (Bahr et al., 2015; Westbrook et al., 2015).

G. salicornia, locally known as "gorilla ogo," was introduced to Kāne'ohe Bay and Waikīkī in the 1970's for aquaculture experiments (Smith et al., 2004). Since then it has expanded its range over 5km from its points of introduction, all the way to the island of Moloka'i. *G. salicornia* forms complex, tenacious mats on coral reefs, which reduce water flow, trap sediments, decrease irradiance, and smother benthic corals (Smith et al., 2002; Martinez, 2012).

While many reef algae are ephemeral and may be well represented by a 20 day experiment, *G. salicornia* has a long residence time on the reef, and thus may have impacts on the coral community that cannot be extrapolated from short term experimental exposures. This study seeks to identify the effects of longer-term exposure to *G. salicornia* using *Porites lobata*, a pan-Pacific species and a commonly occurring reef builder on Hawaiian reefs.

3.3 Methods

Porites lobata, a widely distributed reef-building coral, was collected from Kewalo Basin, on the south shore of O'ahu, Hawai'i via SCUBA, under DAR Special Activity Permit number 2016-66 (Figure 3.1). Five small nubbins were collected with a hammer and chisel from each of 10 visually healthy parent colonies. The first piece from each colony was immediately (within 2 minutes of contact) flash frozen in liquid nitrogen as a baseline sample (N=10). Remaining nubbins were transported in seawater to a shaded, flow-through seawater table at Kewalo Marine Lab, where they were trimmed and affixed to ceramic plugs (http://www.oceanswonders.com/ceramics.html) with cyanoacrylate superglue. Plugs were pre-labeled with letters that would randomly assign one nubbin from each colony to each of 4 groups: 30d exposure, 60d, 85d exposure, and control (N=10 for each). Labeled nubbins were allowed to acclimate and heal for two months before the exposure experiment. Over this time rapid lesion healing and new growth were observed, and all nubbins regained normal coloration before the beginning of the experiment.

Mats of *G. salicornia* were collected from Waikīkī, O'ahu via snorkel and transported to Kewalo Marine Lab in seawater. Mats were cleaned of sediment and epiphytes prior to being shaped into approximately 6 x 4 inch rectangular experimental mats without cutting or breaking algal thalli. Experimental mats were secured with fishing line onto plastic lighting (eggcrate) platforms (Figure 3.2) and allowed to acclimate and heal in an isolated flow-through seawater tank for one month prior to the start of the experiment.

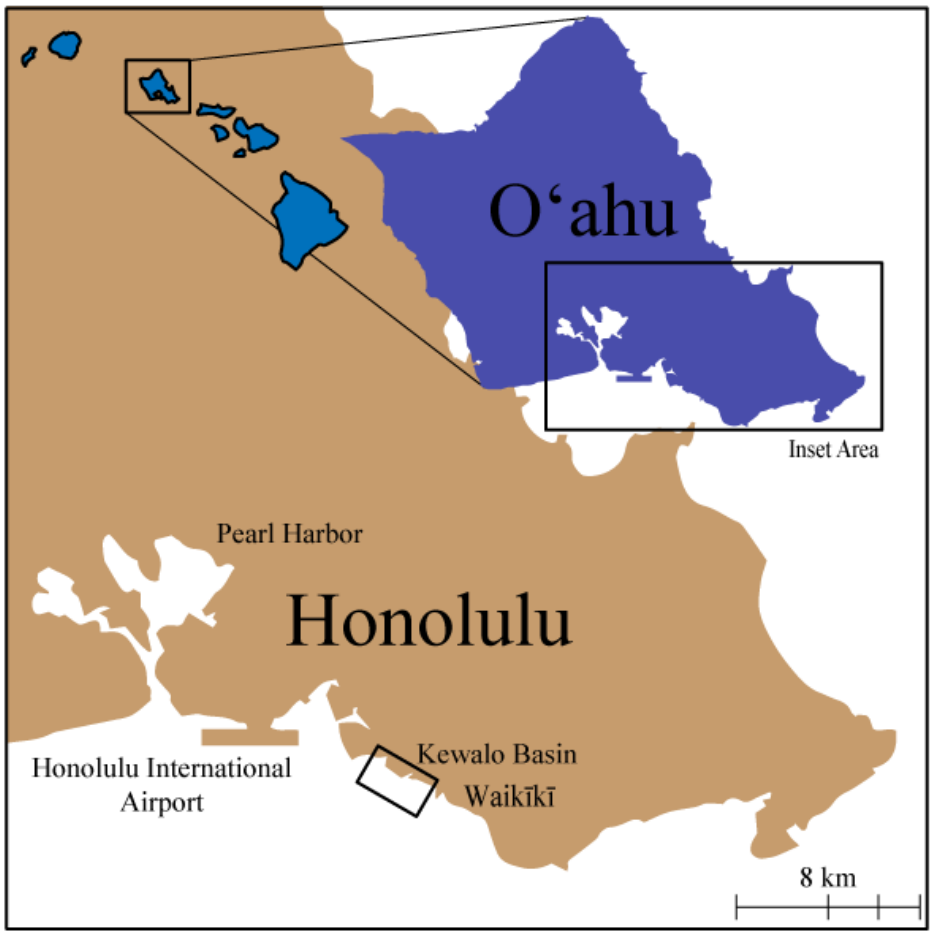

FIGURE 3.1: Map of Collection Site. *Porites lobata* nubbins were collected from Kewalo Basin (boxed). *Gracilaria salicornia* mats were collected off of Waikīkī beach.

3.3.1 Algal exposure experiment

After coral nubbins and algal mats were acclimated, coral nubbins were randomly distributed (not grouped by treatment or colony) between experimental algal mats and placed in direct side-by-side contact with the alga, about 12 nubbins per mat (Figure 3.2). Experimental arrays of algal mats were maintained in one common, isolated flow-through seawater system with natural seawater (passed through a coarse filter sock and bubble trap) in an outdoor seawater table, covered with a 80% shade cloth canopy. The control (no direct contact with algae) coral nubbins were placed on a separate rack, upstream of the experimental racks to reduce exposure to algal exudates.

After an exposure period of 30, 60, or 85 days (hereafter: 1, 2, and 3 months), one nubbin from each colony (N=10) was collected and immediately flash frozen in liquid nitrogen and stored at

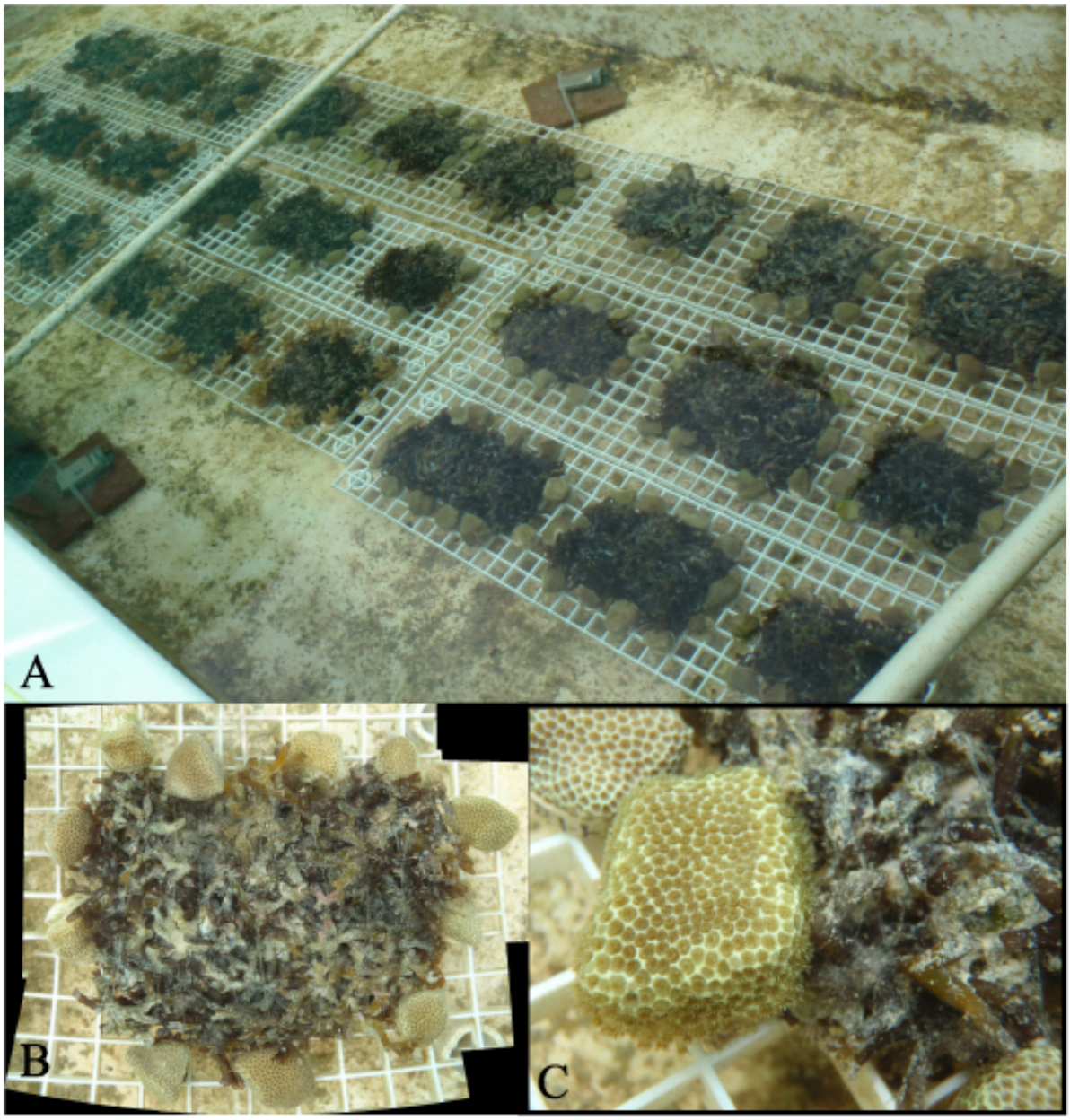

FIGURE 3.2: Algae exposure experiment tank setup. Rectangular mats of *G. salicornia* were surrounded by *P. lobata* nubbins on lighting eggcrate racks (B). All nubbins maintained direct contact with the algal mats throughout the experiment (C). Experimental racks were maintained downstream of the control nubbin rack, out of frame (A).

-80°C until analysis. All control nubbins were collected and frozen at 85 days with the 3-month exposure treatment nubbins.

3.3.2 Bouyant weight and visual health

Beginning immediately after nubbins were permanently affixed to ceramic plugs and continuing weekly through acclimation and exposure, each nubbin was weighed using the bouyant weight technique (Jokiel et al., 1978), photographed, and assigned a qualitative visual health score (normal, partial pale, pale, partial bleached, bleached, partial mortality, full mortality). This health score was for the nubbin in its entirety, so if only the side facing and in contact with the alga was discolored, then the nubbin would be scored with partial paling or bleaching.

Weekly growth rate was calculated as final weight - initial weight, standardized to initial weight. Growth rate data were square root transformed to a base of 0.01 to achieve normality and homoscedasticity, as determined by the Shapiro-Wilk normality test ($p = 0.1$) and Henry's q-q plot. An ANOVA F-test was run on a linear model of growth rate by treatment*month*colony, with Tukey's post-hoc analysis. All nubbins were distributed throughout the tank during the acclimation phase, which is counted here as a pre-treatment. After exposure began, nubbins were separated into treatment and control groups.

Proportions of nubbins with each visual health score were analyzed by treatment over time using an asymptotic generalized Cochran-Mantel-Haenszel test of health score by treatment, stratified by month (1, 2, 3 month), part of the "coin" package in R (Horhorn et al., 2008).

3.3.3 Total lipid analysis

Total lipid quantity was measured using the methods of Stimson (1987). A small chunk of coral (0.20 - 0.40 g) was removed from each frozen nubbin and placed in a solution of 10% Z-fix in milliQ water for 24 hours. Nubbins were rinsed twice in milliQ water and placed in a 2:1 chloroform:methanol solution for 24 hours at room temperature to extract lipids. This solution was filtered through coarse filter paper and the vial, nubbin, and paper were rinsed through with two additional volumes of chloroform:methanol solution into empty, pre-weighed scintillation vials. Vials were then evaporated at 60°C overnight, cooled, and weighed. After extraction of lipids, coral nubbins were decalcified in 10% HCl for 24 hours, rinsed in milliQ water, dried at 60°C, cooled, and weighed.

Total lipid content was calculated as percent lipid (extracted lipid dry weight / (decalcified tissue dry weight + extracted lipid dry weight)) * 100 and compared between treatments and colonies (after square root transformation to achieve normality. Shapiro-Wilk test: $p = 0.07$), by running an ANOVA F-test on a linear model of lipid content by treatment + control, with Tukey's post-hoc analysis.

3.3.4 Zooxanthellae density

Zooxanthellae density was determined using the methods of Martinez et al. (2012). Frozen coral nubbins were crushed into a powder using a hydraulic press. About 0.3g of the resulting powder was transferred to a 2mL tube with 1mL of ice cold filtered seawater (FSW) and homogenized with a tissue tearor for 1 minute on ice, resulting in an emulsified tissue slurry. The tissue tearor was rinsed through with 500µL FSW and washed with ddH_2O, 70% ethanol, and FSW between samples.

The samples of tissue slurry were mixed by vortex for 30 seconds, then allowed to sit on ice for 2 minutes to settle. Supernatant (including zooxanthellae) was transferred to labeled 15 mL tubes. Remaining powder was rinsed with 1 mL ice cold FSW as above three more times. 15 mL tubes of zooxanthellae-containing supernatant were centrifuged at 3,000g for 5 minutes in a refrigerated centrifuge (4°C). Supernatant was discarded and the remaining zooxanthellae pellet was resuspended with 100 µL FSW. 100 µL of resuspended zooxanthellae were removed for zooxanthellae density counts, transferred to a 1.5 mL tube, spun down, and resuspended with 100 µL 4% Z-fix solution (in FSW) to fix zooxanthellae for counting. The remaining 900 µL of resuspended zooxanthellae was transferred to a 2 mL tube, spun down, and frozen dry at -80°C for pigment analysis.

Fixed zooxanthellae were counted twice in a Neubauer hemocytometer (in 10 µL portions). For each count, all cells in 5 squares (4 corners and the center square) were counted. If the zooxanthellae cell count was less than 100 after counting 5 squares, all 9 were counted. Zooxanthellae density was calculated as cells per mL per gram of starting material and compared between treatments and colonies with an ANOVA F-test (after square root transformation to achieve normality. Shapiro-Wilk test: $p = 0.7$) of zooxanthellae density by treatment + colony.

3.3.5 Zooxanthellae pigment composition

Frozen zooxanthellae pellets were resuspended in 100 µL 90% acetone and incubated at 4°C for 24 hours. Samples were mixed by vortex, spun down on a mini centrifuge, and loaded in triplicate into an optically clear 96-well plate. Absorbance was read at 480, 510, 630, and 664 nm. Concentration of chlorophyll A, chlorophyll C2, and carotenoids were calculated using the equations from Jeffrey and Welschmeyer (1997) and compared between treatments and colonies using ANOVAs after log10 transformation to achieve normality (Shapiro-Wilk test $p = 0.6$), with Tukey's post-hoc analysis.

All statistical analyses were performed in R.

3.4 Results

3.4.1 Growth Rate

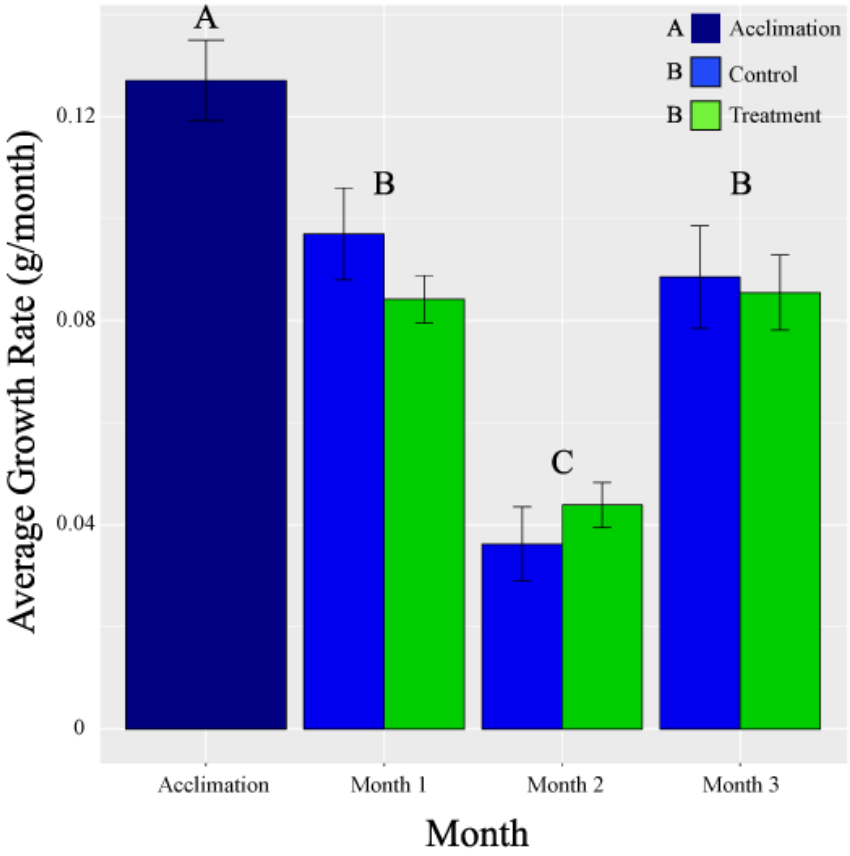

FIGURE 3.3: Average coral growth rate (in grams buoyant weight per month) by treatment over time with SE bars. Growth during acclimation was significantly higher and growth during month 2 significantly lower regardless of treatment after exposure ($p<0.001$). Tukey's post-hoc test groups distinguished by letters. Those over the bars correspond to differences over time (acclimation through month 3), while those by the legend correspond to differences between treatments.

All nubbins were approximately 2.5 cm in diameter when they were permanently affixed to ceramic plugs. Change in buoyant weight was calculated as growth rate in grams per month. Coral nubbins grew quickly, healing cut edges and in most cases growing onto the ceramic plug by the end of the acclimation phase.

An ANOVA testing the effects of treatment, month, colony and their interactions on growth rate revealed significant differences in growth rate between months of the experiment ($F_{2/205}=37.88$, $p<0.001$), with the acclimation month as the highest, the second month of exposure as the lowest,

and months 1 and 3 of the exposure clustering together in-between (Figure 3.3). The significant difference between treatments ($F_{2/205}$=18.52, p<0.001) was revealed by Tukey's post-hoc analysis to be due to the higher growth rate during acclimation, with no significant difference between control and treatment groups after algae exposure, and no significant interaction between treatment and month ($F_{2/205}$= 0.75, p= 0.47).

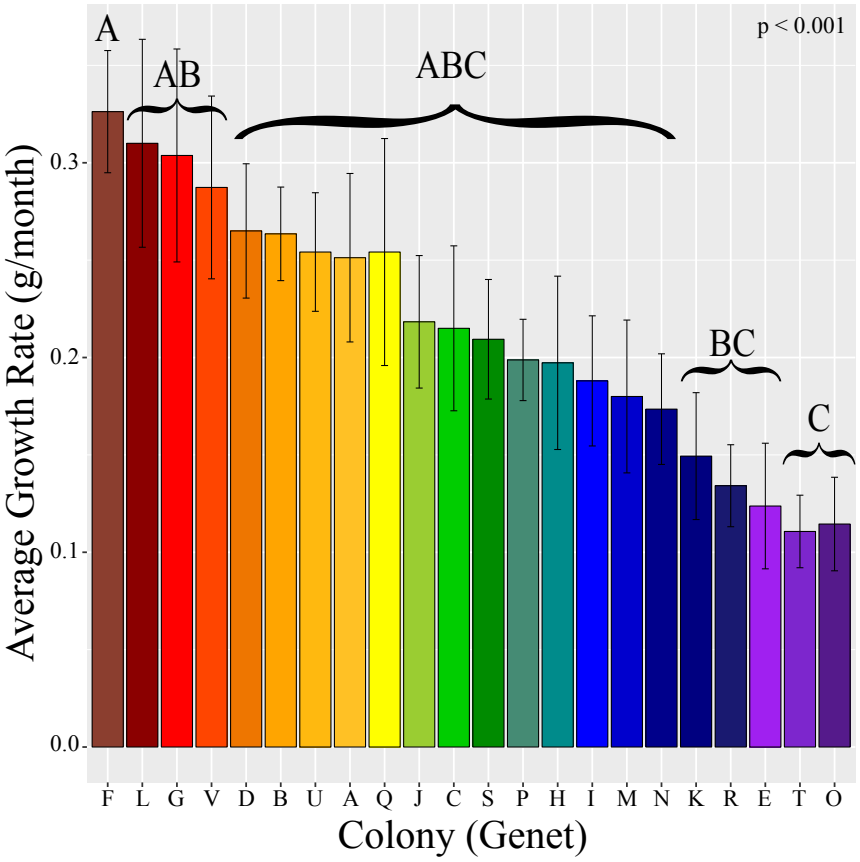

FIGURE 3.4: Average coral growth rate in grams buoyant weight per month by colony with SE bars. Lettered groupings were significantly different (p<0.001).

There was a large amount of variation in growth rate between individual coral colonies, which had significantly different growth rates over the course of the experiment ($F_{21/205}$= 6.79, p<0.001). The 22 colonies (4 nubbins per colony) clustered into five statistically distinct groups using Tukey's post-hoc analysis at alpha=0.05 (Figure 3.4), and there was a significant interaction between coral colony and treatment ($F_{34/205}$= 2.70, p<0.001), with some colonies responding more strongly to algae exposure than others.

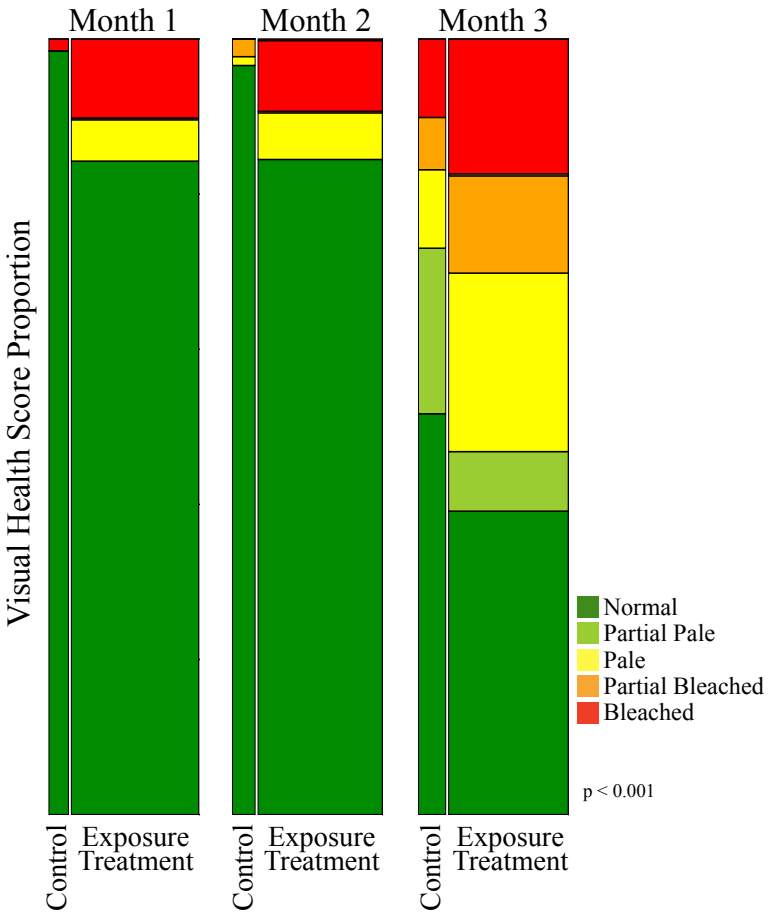

FIGURE 3.5: Visual health of *Porites lobata* nubbins by treatment (exposure vs. control) over time. Significantly more bleaching was observed between control and exposure nubbins for all time points (p<0.001).

3.4.2 Loss of zooxanthellae

Loss of zooxanthellae was evaluated in three ways, 1) through qualitative visual health scores, 2) with quantitative zooxanthellae density counts, and 3) by measuring the abundance of photosynthetic pigments present in extracted zooxanthellae.

Visual health scores (Normal, Partial Pale, Pale, Partial Bleached, Bleached, Partial Mortality, Mortality) were assigned to each nubbin during weekly weighing. Proportions of scores for each treatment and month were analyzed with the Asymptotic Generalized Cochran-Mantel-Haenszel test, which showed that these proportions were significantly different between treatments and months ($X^2 = 46.87$, df=10, p<0.001). For each month, the algae exposure treatment displayed significantly more pale and bleached tissue than the control (Figure 3.5).

These findings were not reflected in the zooxanthellae density counts. Zooxanthellae per

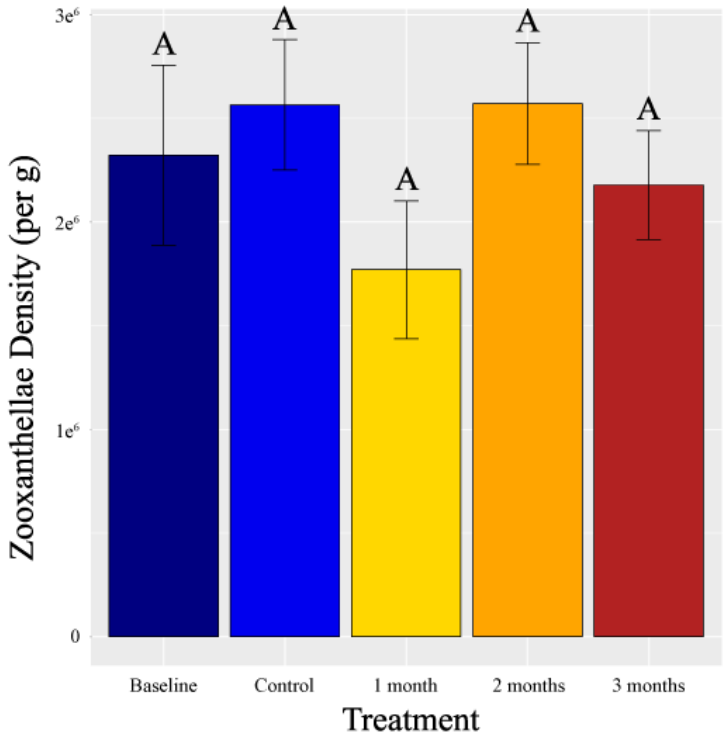

FIGURE 3.6: Zooxanthellae density in cells per gram of coral tissue for all time points of exposure (1,2,3 month), control and baseline with SE bars. No significant difference was found between treatments (p=0.28).

mL suspended sample per gram of starting crushed coral tissue did not differ significantly between any of the treatments (Baseline, Control, 1 month exposure, 2 month exposure, 3 month exposure; $F_{4/36}$=1.36, p=0.27; Figure 3.6) or colonies $F_{9/36}$=1.12, p=0.38 (Supplemental Figure 1).

Rather than a change in zooxanthellae density, the observed paling seems to have resulted from a decrease in photosynthetic pigments of the zooxanthellae. Significant differences were detected in pigment content between treatments for chlorophyll a ($F_{4/36}$=2.67, p=0.05) and chlorophyll c2 ($F_{4/36}$=3.68, p=0.01), with less of a difference detected for carotenoids ($F_{4/36}$=2.25 ,p=0.08). Tukey's post-hoc analysis grouped the baseline samples as the highest, 1 month exposure samples as the lowest, and the control, 2 month, and 3 month samples together as intermediate for all pigments (Figure 3.7). There were no significant differences in pigment content between colonies for chlorophyll a ($F_{9/36}$=0.84, p=0.59), chlorophyll c2 ($F_{9/36}$=1.31, p=0.26), or carotenoids ($F_{9/36}$=0.98, p=0.47).

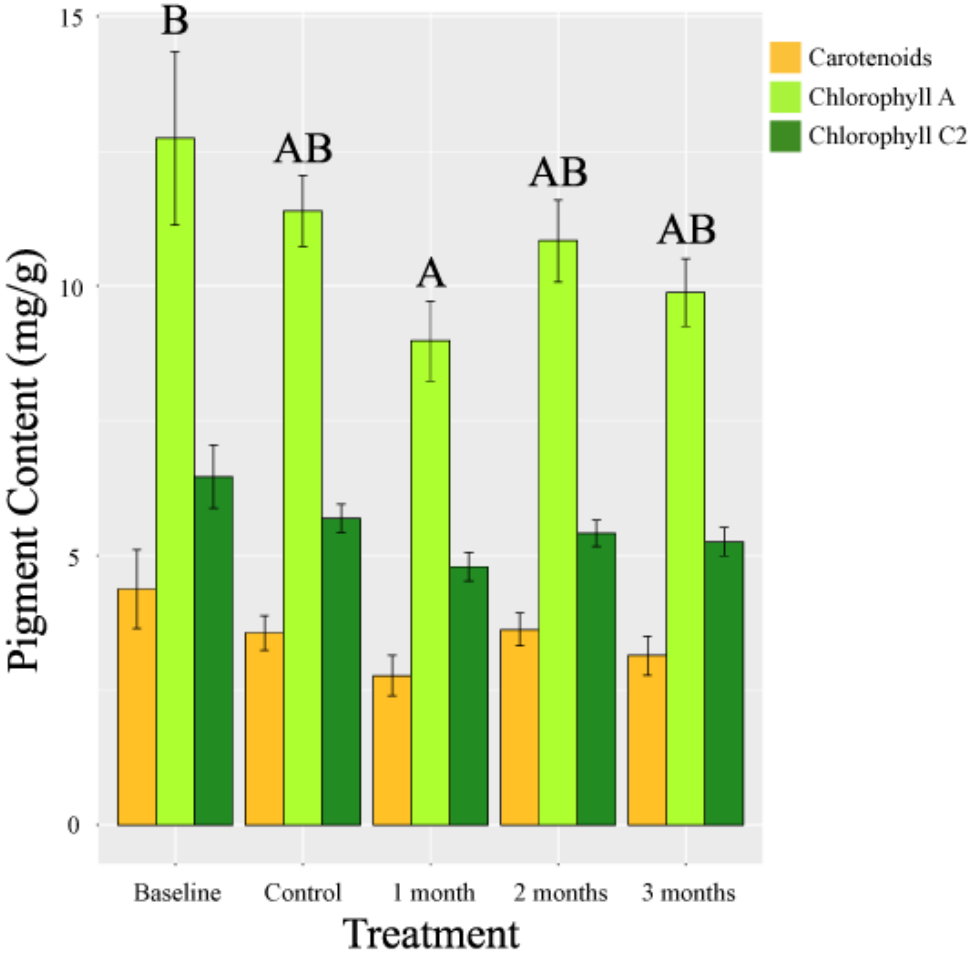

FIGURE 3.7: Pigment content analysis of *Porites lobata* nubbins. Chlorophyll a, chlorophyll c2, and carotenoid content shown in mg/g for all timepoints of exposure (1,2,3 month), control, and baseline nubbins, with SE bars. 1 month exposure was significantly lower and baseline significantly higher in both chlorophyll a and c2 (p=0.05, p=0.01, respectively).

3.4.3 Total lipid content

The percentage of total dry tissue weight made up by lipids represents the stored energy available to the coral animal and can be used as a proxy measure for coral animal metabolism (Rodrigues and Grottoli, 2007). A one-way ANOVA comparing percent total lipids present in coral tissue between treatments showed that there was a significant difference ($F_{4/36}$=3.42, p=0.02), with the highest average percentage of lipids present in the first month of exposure (44.06%, Figure 3.8). Percent lipid in coral tissue did not differ significantly between colonies ($F_{9/36}$=1.51, p=0.12, Supplemental Figure 2).

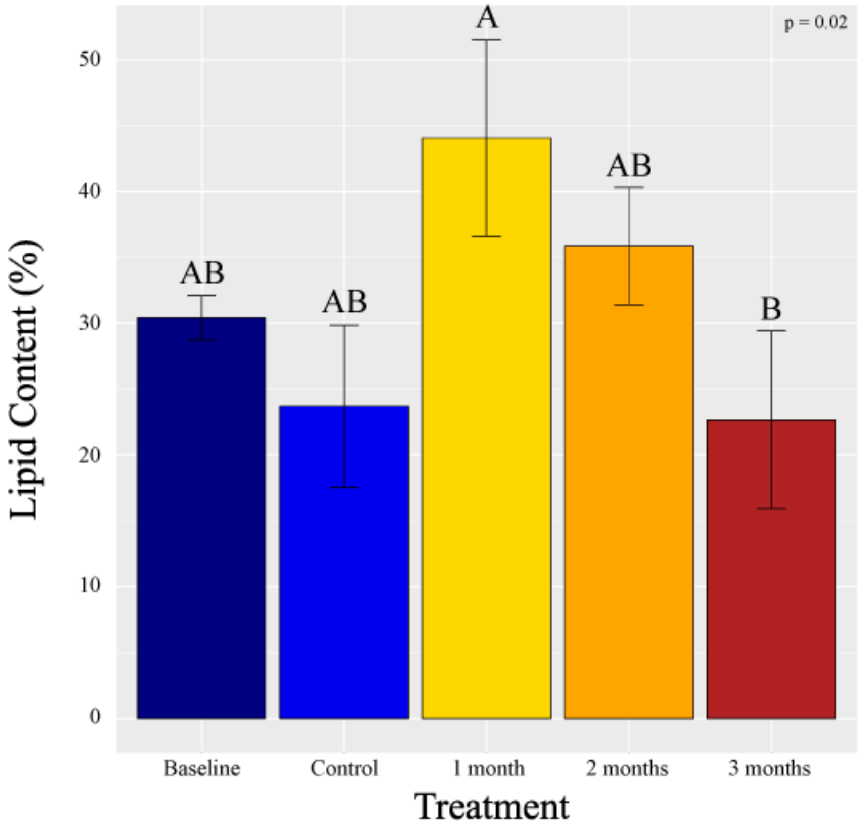

FIGURE 3.8: Total lipid content (% dry weight) of *P. lobata* nubbins by treatment with SE bars. Letters correspond to groupings produced by Tukey's post-hoc analysis (p = 0.02).

3.5 Discussion

Replicates of 10 colonies of *Porites lobata* were placed in direct contact with the invasive red alga, *Gracilaria salicornia* for 30, 60, and 85 days to determine the effect of this common stressor on the physiological health of the most common and important reef-building coral in Hawai'i.

3.5.1 Loss of Pigment

Bleaching (partial to full loss of coral color) is a common stress response of corals and a commonly used proxy for coral animal health (Siebeck et al., 2006). In keeping with the findings of previous studies, the coral nubbins exposed to algal mats during this experiment displayed increased prevalence and intensity of bleaching compared to those corals not in contact with algae. However, this was not reflected in a corresponding decrease in zooxanthellae density as might be expected. Rather it seems the quality, not the quantity of zooxanthellae was affected by algal contact, seen as a significant decrease in concentration of all three zooxanthellae pigments

tested: chlorophyll a, chlorophyll c2, and carotenoids. While zooxanthellae density has been seen to vary by species, depth, and between parts of a single colony (Helmuth et al., 1997), no significant difference was seen in zooxanthellae density between colonies of *P. lobata* over the course of this study.

This study differs from a previous experiment involving *G. salicornia* mats in that coral nubbins in this study were placed in direct contact with, but not underneath, mats of algae and therefore were not expected to experience whole-nubbin shading, which could result in a dark bleaching response (Martinez, 2012). Some coral nubbins in this experiment were observed to be more pale or bleached on the side of the coral that was in direct contact with (and partially shaded by) the algal mat, while many others experienced nubbin-wide patchy or full paling or bleaching. The heightened bleaching response and reduction in photosynthetic pigment concentration observed in coral nubbins directly in contact with algae during this experiment may have resulted from a variety of mechanisms previously linked to coral bleaching including: partial shading (McCook et al., 2001; Martinez et al., 2012) allelopathic interactions (Rasher and Hay, 2010), and coral microbiome disruption (Smith et al., 2006). Further studies would be needed to determine the exact mechanisms at play in these specific interactions between *G. salicornia* and *P. lobata*.

3.5.2 Altered Metabolic State

In this experiment, *Porites lobata* nubbins increased their total lipid content in the first month after exposure, relative to both the baseline and control samples. After two months of exposure there was no significant difference in algae-contact nubbins compared to either baseline or control samples. After three months (85 days) of exposure, total lipid content was significantly reduced compared to the first month of exposure, but still not significantly different from the baseline, control, and 2 month exposure samples.

Loss of photosynthetic potential through the loss of either zooxanthellae or zooxanthellae pigments reduces the amount of sugar available to the coral animal and represents a substantial loss of energy because fully functioning zooxanthellae can provide coral hosts with over 100% of their daily metabolic energy requirements (Muscatine and Cernichiari, 1969). Stored excess energy in the form of lipids may make up 30-40% of coral tissue, 26% of which is structural (Stimson, 1987). These coral energy reserves are large enough that it has been estimated that *Porites lobata* colonies store enough lipid energy at any time to sustain the coral animal for an estimated 71 days (Grottoli et al., 2004). Previous studies have found that total lipid content is negatively correlated with light level (Stimson, 1987) and positively correlated with zooxanthellae density (Yamashiro et al., 2005) and tracks closely with chlorophyll a concentration (Rodrigues and Grottoli, 2006, 2007), yet some massive coral colonies have been observed to survive bleaching

events without major losses to their lipid stores (Yamashiro et al., 2005), for example *Montipora capitata* has been seen to recover lipid stores post-bleaching faster than it recovers chlorophyll a concentrations or zooxanthellae density (Rodrigues and Grottoli, 2006).

As a massive, stress-tolerant coral (Darling et al., 2012), *Porites lobata* may be more robust to lipid store depletion that might be expected to accompany the observed decrease in photosynthetic pigments. It is also possible that responding to the stress of direct contact with algal mats did not require any change in coral animal energy budgets, especially because zooxanthellae density was not significantly affected by contact with *G. salicornia*. This is supported by the fact that growth rate did not differ significantly after exposure. The initial increase in percent lipids (Figure 3.8) combined with the unaffected growth rate (Figure 3.3) may suggest that the zooxanthellae were still able to supply sugars in excess of what the coral animal required, despite the observed bleaching response, or that the coral animal was able to make up it energy requirements through increased heterotrophic feeding. Further research into *Porites lobata* energy budgets under stress would go a long way towards expanding our understanding of the physiological effects suffered by important reef builders due to environmental stressors.

3.5.3 Tolerant Coral and Poorly Defended Alga

The sub-lethal, temporary response elicited from *P. lobata* by *G. salicornia* during this experiment may be due to the specific pairing of a hardy, stress-tolerant coral with a poorly defended alga.

G. salicornia is not known to be highly chemically defended. While red algae (phylum Rhodophyta) tend to be particularly rich in biologically active compounds (Hay and Fenical, 1988), the genus *Gracilaria* is characterized by moderately to highly palatable species (Duffy and Hay, 1990; Cetrulo and Hay, 2000; Erickson et al., 2006; Littler and Littler, 2007). Several studies have observed antifungal and antimicrobial activities of *Gracilaria spp.* extracts (Engel et al., 2006; Puglisi et al., 2007), and *G. conferta* has been observed to react to tissue damage with bursts of reactive oxygen species (Potin et al., 2002), but these do not seem to translate to strong chemical defenses against either herbivory or coral contact (Martinez, 2012). *Gracilaria* species are commonly more fouled by epiphytes than other more defended species (Schmitt et al., 1995) and have mild if any effect on survivorship and settlement of larvae including those of tubeworm *Hydroides elegans*, bryozoan *Bugula nerita*, and the coral *Pocillopora damicornis* (Walters et al., 2003; Martinez, 2012).

P. lobata is known to be a highly stress tolerant coral (Darling et al., 2012), robust against bleaching stress (Baker et al., 2004; Darling et al., 2013) and algae contact stress (Bulleri et al., 2013; Bonaldo and Hay, 2014). Other common Hawaiian coral species, such as the weedy *Pocillopora damicornis* or competitive *Montipora capitata* (Darling et al., 2012), both of which are much

less stress tolerant than *P. lobata*, have been seen to have different responses to the same *G. salicornia* contact stressor (unpublished data). It is common for coral of different genotypes, morphologies, and life history strategies to react very differently to common stressors, including macroalgae (Bulleri et al., 2013; Bonaldo and Hay, 2014).

3.5.4 Colony and Tank Effects

It is interesting to note that in this study, which used the same 10 coral colonies in each treatment, there were not significant differences between coral colony responses when testing zooxanthellae density, percent lipid, or pigment content (Supplemental Figures A.1, A.2).

Significant differences in growth rate were observed over the course of the experiment (Figure 3.3), along with a corresponding overall increase in bleaching (Figure 3.5), suggesting a tank-wide effect not related to the experimental algal exposure. The experiment was run from May through August, 2016, a year that did not experience a bleaching event in Hawai'i. While the tank was shaded with an 80% shade cloth and supplied with natural seawater from a constant flow-through ocean pump, the shallow outdoor tank was susceptible to ambient temperature fluctuations. Figure 3.9 shows average temperature and irradiance from six Hobo data loggers placed throughout the tank during the experiment. Unfortunately the time series ends before the end of the experiment, but based on these data, ANOVA tests indicate that average temperature was significantly different between months of the experiment ($F_{1/93}$= 249.41, p<0.001), steadily rising throughout the summer, while average irradiance did not vary significantly over the course of the experiment ($F_{1/93}$= 0.07, p= 0.79).

The increased overall paling and bleaching observed over the course of the experiment (Figure 5) was likely due to this increased temperature. However, regardless of this overall increase, those corals exposed to algae still displayed higher bleaching and partial mortality than those not exposed to algae.

This steady increase in temperature throughout the experiment does not explain the pattern observed for growth rate (Figure 3.3), which dips to its lowest in month 2 (July), whereas the hottest month is month 3 (August). But it does track closely with the observed change in lipid content (Figure 3.8), which steadily declines throughout the experiment. It is possible that the effect of algal contact on the lipid content of *P. lobata* is confounded by effects of temperature on this same parameter. However, the effect of temperature alone on coral energetics has yet to be determined.

3.5.5 Conclusion

Corals experiencing direct contact with algal mats exhibited higher prevalence and severity of bleaching, reduced photosynthetic pigment concentrations, and altered lipid content. However,

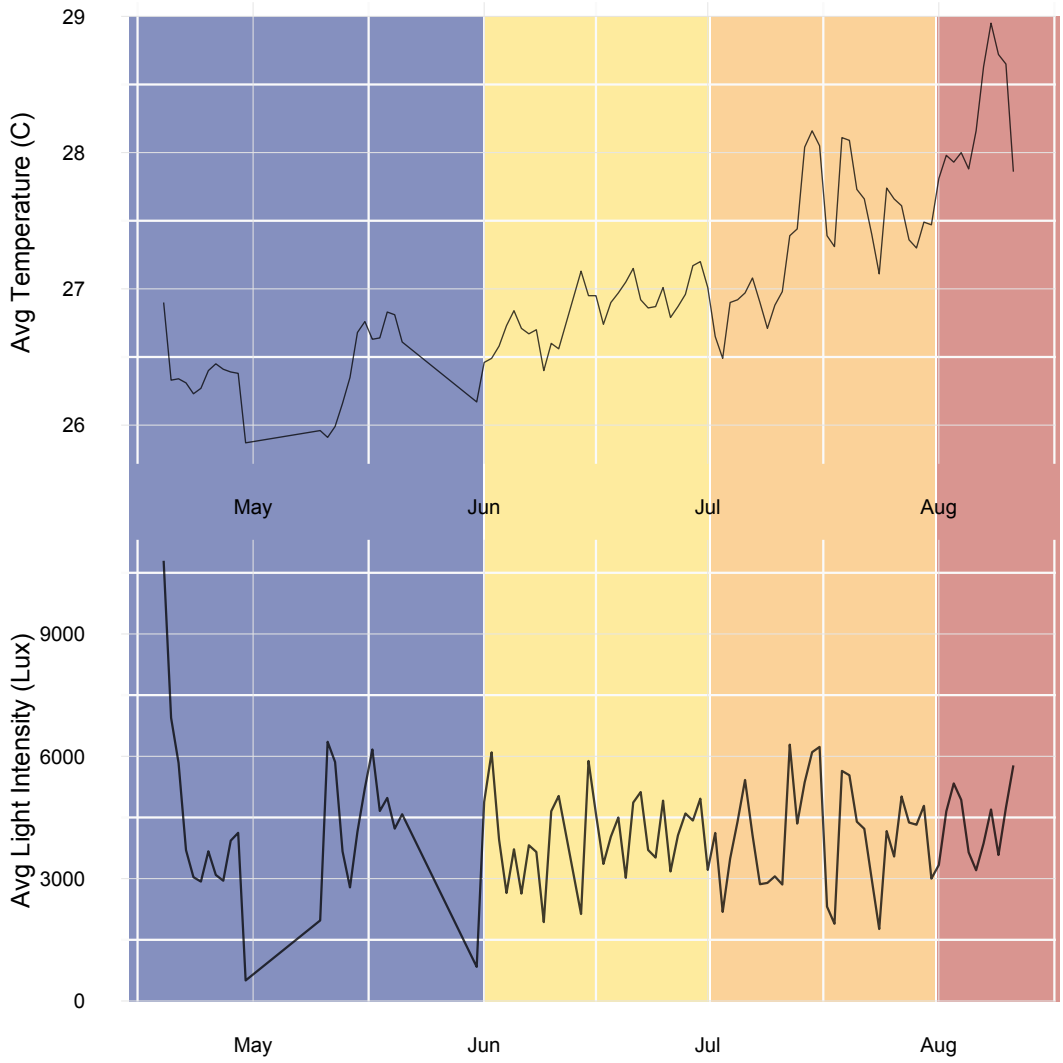

FIGURE 3.9: Tank conditions over the course of the experiment from Hobo data loggers. Average temperature (top) varied significantly over the course of the experiment ($p<0.001$). Average irradiance (bottom) did not ($p= 0.79$). Graph is shaded to display phases of the experiment (acclimation = blue, month 1 = yellow, month 2 = orange, and month 3 = red).

contact with *G. salicornia* mats seems to have had a sub-lethal and temporary impact on *P. lobata* during this experiment. For all physiological metrics used in this study, the 30 day exposure treatment was the most dissimilar to the control group, suggesting that the response of this coral to algal contact is more immediate and acute rather than chronic, even if the exposure persists as in the case of long-lasting, invasive algal mats. The fact that the 3-month exposure treatment groups away from the 1-month exposure and often with the control or baseline treatment suggests that there is a fairly rapid acclimation response of the coral to algal contact, though the response of less tolerant corals common on Hawaiian coral reefs is almost certainly more severe

than that of stress-tolerant *P. lobata*.

While this 3 month exposure represents a longer duration than the majority of laboratory coral-algae interaction experiments, which typically last less than 20 days, it still represents a relatively short time on ecological time scales. Given time under the same conditions, the *G. salicornia* mats would have completely overgrown the *P. lobata* nubbins. So while the results of this experiment indicate that *P. lobata* may not be as directly impacted by direct contact with the invasive algal mats as suggested by previous short-term experiments, the conditions of the experiment were such that they are only directly applicable to situations in which the coral and alga are in contact without the algal mat growing directly over the coral - a condition which is only maintained in areas of high herbivory or water motion.

Chapter 4

Conclusion

4.1 Summary

The shallow reefs around O'ahu, Hawai'i are affected by high recreational use, reduction of herbivores, nutrification, pollution, sedimentation, and alien algae invasions (Hunter and Evans, 1995; Smith et al., 2002; Wolanski et al., 2004; Cote et al., 2005; Birkeland, 2015; Rodgers et al., 2010). These stressors contribute to the decreased health, growth, and reproduction of reef-building corals, and favor the proliferation of fast-growing macroalgae (Hatcher and Larkum, 1983; Littler and Littler, 1984; Stimson et al., 2001; Smith et al., 2001). This trend is not unique to Hawai'i, reduced coral cover and increased biomass of benthic macroalgae have been reported in areas of all ocean basins (Gardner et al., 2003; Bellwood et al., 2004; Cote et al., 2005; Bruno and Selig, 2007; Hughes et al., 2007; Sweatman et al., 2011; Chadwick and Morrow, 2011; Mumby and Steneck, 2011; De'Ath et al., 2012). The increased prevalence of macroalgae on coral reefs necessarily brings coral and algae into closer and more frequent contact as they compete for space and light, a trend that is reflected in the increased focus on coral-algal interactions in coral reef research (Barott and Rohwer, 2012).

The research presented in this thesis was designed to fill in missing information needed to understand the state of coral-algal interactions around Hawaiian reefs, and to address gaps identified in the coral-algal interaction literature. While many manipulative experiments (exposures and removals) have been performed to test the specific effects of algae on coral and vice versa (Smith et al., 2006; Rasher and Hay, 2010; Rasher et al., 2011; Morrow et al., 2012a,b; Longo and Hay, 2015), the vast majority last less than 20 days and naturally-occurring interactions are rarely surveyed (Haas et al., 2009; Bonaldo and Hay, 2014; Bruno et al., 2014). This thesis reports the findings of the first coral-algal interaction survey for the Hawaiian islands as well as a 3-month exposure experiment testing the effects of *Gracilaria salicornia* on *Porites lobata*.

The observed patterns of coral-algal interactions naturally occurring around the island of O'ahu, Hawai'i suggest that the surveyed reefs are not heavily impacted by macroalgal growth. 47% of corals surveyed were in direct contact with at least one macroalga, with an observed interaction rate of 0.28 interactions per coral colony (2.2 per meter^2). This rate is comparable those reported during the low algal abundance season in the Red Sea (Haas et al., 2009), and much lower than that reported around Heron Island, Australia (Tanner, 1995). The interactions varied

in severity, with 33% of corals in these interactions displaying normal color and tissue, 19% of corals showing discolored tissue, 14% looking pale, and 16% experiencing direct overgrowth of algae. Only 3% of interactions surveyed were characterized by bleached tissue around the area of contact, and only 6% showed signs of tissue abnormality or mortality. However, these surveys represent only a snapshot of coral-algal interactions and therefore cannot predict or report on the ultimate outcomes of these interactions.

As expected, the rate and severity of interactions differed significantly by coral species, though the patterns were somewhat surprising with stress tolerant *P. lobata* exhibiting much more severe responses to algae contact than competitive and weedy species such as *Pocillopora damicornis* and *Montipora capitata*. The rate and severity of interactions also differed significantly by site, with the protected Marine Life Conservation District sites (Pūpūkea and Hanauma Bay) having the fewest observed interactions, but Kaiona (a heavily used and unprotected area of the windward coast) having the least severe interactions. Investigations into environmental parameters that may influence these differences in site-level severity suggest that none of the predictors investigated are heavily influencing the observed patterns, but that sea surface temperature anomalies are the most correlated with site-level severity differences in interactions involving *P. lobata*.

The 3-month exposure of *P. lobata* nubbins to mats of the invasive *G. salicornia* shows a pattern of stress response that suggests the effect of contact between these two competitors is relatively mild and acute, with signs of acclimation after 3 months of exposure. In keeping with previous short-term studies, those coral nubbins exposed to algal mats exhibited higher prevalence and severity of bleaching, reduced photosynthetic pigment concentrations, and altered lipid content. None of the nubbins in either the control or the treatment groups died during the course of the experiment, which signifies a much more mild response than many reported in the coral-algal interaction literature (Rasher and Hay, 2010; Rasher et al., 2010, 2011; Smith et al., 2006). For almost all physiological metrics used in this study, the 30-day exposure treatment was the most dissimilar to the control group, and in most cases the 85-day exposure treatment groups with the control or baseline nubbins. This suggests an acute, temporary response followed by a fairly rapid acclimation response.

4.2 Implications and Recommendations

The results presented here provide needed baseline data, highlight several avenues for further research, and suggest courses for management of coral reef macroalgae.

The surveys reported in this thesis represent the first quantitative study of naturally-occurring coral-algal research in any of the Hawaiian Islands. While the results are a snapshot look at these interactions, this dataset provides a necessary point of comparison for future studies of coral-algal interactions in the islands. The results show fewer, but not necessarily less severe

interactions in marine protected areas (MPAs) and imply that environmental parameters such as sea surface temperature anomalies and human impacts may explain some of the variation in interaction severity between sites. Further investigations could examine the trends suggested by these data such as the role of MPAs in affecting coral-algal interaction rate and severity, and the environmental conditions that predict the prevalence and severity of interactions. The establishment of permanent transects for long-term monitoring could also track these interactions over time to give a better understanding of the persistence of interactions over time and their long-term outcomes.

The effect of *G. salicornia* on *P. lobata* nubbins was relatively mild and temporary, with no observed mortality. While the coral exhibited an initial response to algal contact, the rapid acclimation suggests that long-term interactions between the two species may be neutral rather than actively negative for the coral competitor. When comparing this experiment to the results of the interaction surveys it is interesting to note that *P. lobata* exhibited more severe reactions to algal contact in naturally-occurring interactions than most other coral species. Repeating this experiment with other coral species would provide an interesting comparison to both experimental and natural interaction severities.

Hawaiian reefs are characterized by high coral cover, low coral and herbivore diversity, and high rates of both endemism and alien species (Kay and Palumbi, 1987; Smith et al., 2002; Allen, 2008; Friedlander et al., 2008). Yet trends identified as contributing to the high underlying resilience of other areas of the Pacific hold up for Hawaiian reefs - namely nutrient limitation (bottom-up control) and lower baseline presence and growth rate of macroalgae (Smith et al., 2004; Roff and Mumby, 2012). The studies reported here indicate moderate rates and severities of coral-algal interaction on shallow reefs around O'ahu, with the main reef-building coral of the region, *P. lobata*, exhibiting a sub-lethal, temporary stress response to contact with the pervasive invasive alga, *G. salicornia*. Roff and Mumby (2012) postulate that reefs of the Pacific would need to be heavily degraded before it would be possible for them to undergo a full phase shift to an alternate stable state of algal dominance, and the results of the research reported here do not point towards a looming threat of algal dominance.

Appendix A

Supplemental Figures

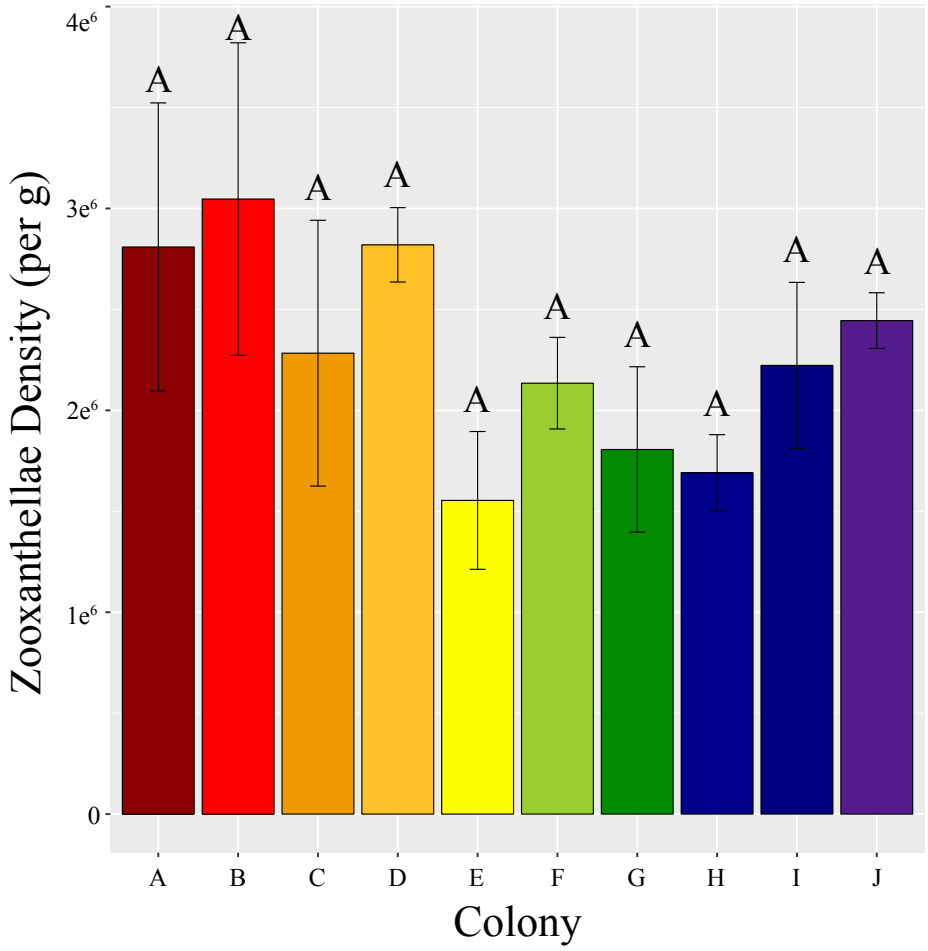

FIGURE A.1: Zooxanthellae density in cells per gram of *P. lobata* coral tissue by colony, with all experimental treatments and time points combined. No statistical differences between colonies were found.

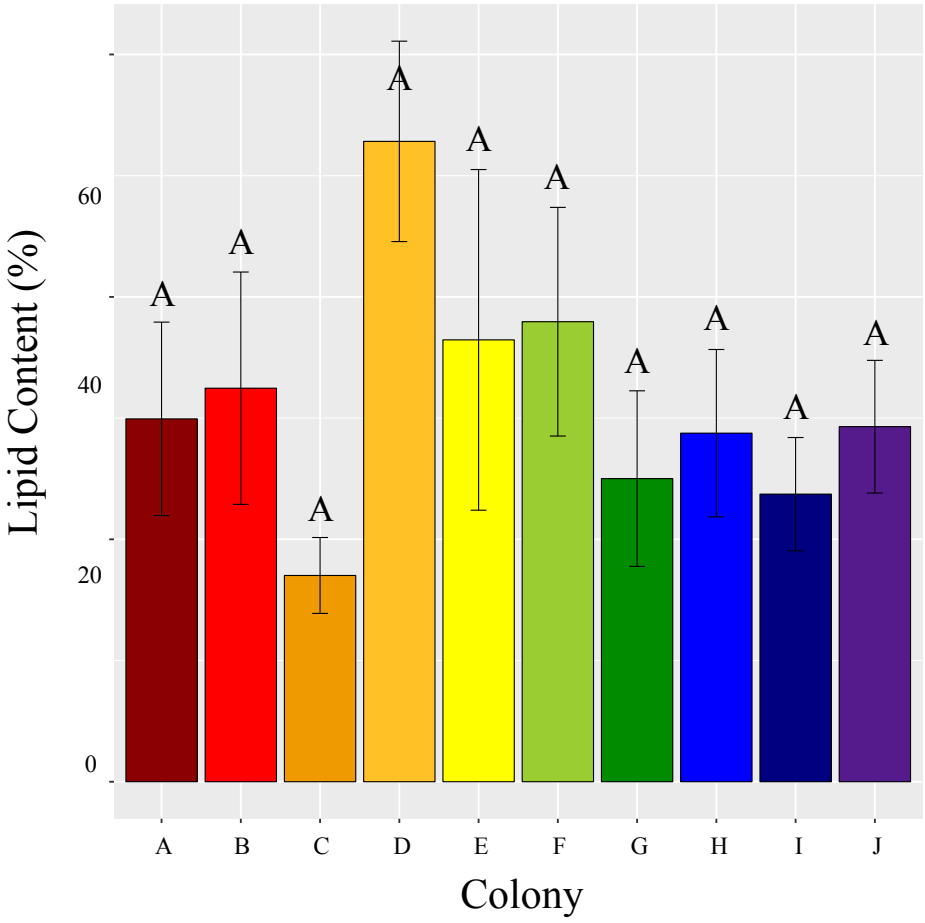

FIGURE A.2: Total lipid content (% dry weight) of *P. lobata* nubbins by colony, with all experimental treatments and time points combined. No statistical differences between colonies were found.

References

Allen, G. R. (2008). Conservation hotspots of biodiversity and endemism for Indo-Pacific coral reef fishes. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 18:541–556.

Angel, D. L., Eden, N., Breistein, S., Yurman, A., and Katz, T. (2002). In situ biofiltration: a means to limit the dispersal of effluents from marine finfish cage aquaculture. *Hydrobiologia*, 469(1-3):1–10.

Ask, E. I., Batibasaga, A., Zertuche-Gonzalez, J. A., and De San, M. (2003). Three decades of *Kappaphycus alvarezii* (Rhodophyta) introduction to non-endemic locations. *Proceedings of the International Seaweed Symposium*, 17:49–57.

Bahr, K. D., Jokiel, P. L., and Toonen, R. J. (2015). The unnatural history of Kāneʻohe Bay: coral reef resilience in the face of centuries of anthropogenic impacts. *PeerJ*, 3(1):e950–26.

Baker, A. C., Starger, C. J., McClanahan, T. R., and Glynn, P. W. (2004). Corals' adaptive response to climate change. *Nature Scientific Reports*, 430.

Barott, K. L., Rodriguez Brito, B., Janouškovec, J., Marhaver, K. L., Smith, J. E., Keeling, P., and Rohwer, F. L. (2011). Microbial diversity associated with four functional groups of benthic reef algae and the reef-building coral *Montastraea annularis*. *Environmental Microbiology*, 13(5):1192–1204.

Barott, K. L. and Rohwer, F. L. (2012). Unseen players shape benthic competition on coral reefs. *Trends in Microbiology*, 20(12):621–628.

Barott, K. L., Williams, G. J., Vermeij, M., Harris, J., Smith, J. E., Rohwer, F. L., and Sandin, S. A. (2012). Natural history of coral–algae competition across a gradient of human activity in the Line Islands. *Marine Ecology Progress Series*, 460:1–12.

Bellwood, D. R., Hughes, T. P., Folke, C., and Nyström, M. (2004). Confronting the coral reef crisis. *Nature Scientific Reports*, 429:827–833.

Birkeland, C. (2015). Geographic differences in ecological processes on coral reefs. In *Coral Reefs in the Anthropocene*, pages 179–194. Springer Netherlands, Dordrecht.

Birrell, C. L., McCook, L. J., Willis, B. L., and Harrington, L. (2008). Chemical effects of macroalgae on larval settlement of the broadcast spawning coral *Acropora millepora*. *Marine Ecology Progress Series*, 362:129–137.

Bonaldo, R. M. and Hay, M. E. (2014). Seaweed-oral interactions: variance in seaweed allelopathy, coral susceptibility, and potential effects on coral resilience. *PLoS One*, 9(1):e85786.

Bruno, J. F. (2013). Coral reefs: building a better crystal ball. *Current Biology*, 23(11):R473–R475.

Bruno, J. F., Precht, W. F., Vroom, P. S., and Aronson, R. B. (2014). Coral reef baselines: How much macroalgae is natural? *Marine Pollution Bulletin*, 80(1-2):24–29.

Bruno, J. F. and Selig, E. R. (2007). Regional decline of coral cover in the Indo-Pacific: timing, extent, and subregional comparisons. *PLoS One*, 2(8):e711.

Bruno, J. F., Sweatman, H., Precht, W. F., Selig, E. R., and Schutte, V. G. (2009). Assessing evidence of phase shifts from coral to macroalgal dominance on coral reefs. *Ecology*, 90(6):1478–1484.

Bulleri, F., Couraudon-Réale, M., de Loma, T. L., and Claudet, J. (2013). Variability in the effects of macroalgae on the survival and growth of corals: the consumer connection. *PLoS One*, 8(11):e79712.

Ceccarelli, D. M., Jones, G. P., and McCook, L. J. (2005). Effects of territorial damselfish on an algal-dominated coastal coral reef. *Coral Reefs*, 24:606–620.

Cetrulo, G. L. and Hay, M. E. (2000). Activated chemical defenses in tropical versus temperate seaweeds. *Marine Ecology Progress Series*, 207:243–253.

Chadwick, N. E. and Morrow, K. M. (2011). Competition among sessile organisms on coral reefs. In Dubinsky, Z. and Stambler, N., editors, *Coral Reefs: An Ecosystem in Transition*, pages 347–371. Springer Netherlands, Dordrecht.

Conklin, E. J. and Smith, J. E. (2005). Abundance and spread of the invasive red algae, *Kappaphycus spp.*, in Kane'ohe Bay, Hawai'i and an experimental assessment of management options. *Biological Invasions*, 7(6):1029–1039.

Connell, J. H. (1978). Diversity in tropical rain forests and coral reefs. *Science*, 199(4335):1302–1310.

Cote, I. M., Gill, J. A., Gardner, T. A., and Watkinson, A. R. (2005). Measuring coral reef decline through meta-analyses. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 360(1454):385–395.

Cronin, G. and Hay, M. E. (1996). Within-plant variation in seaweed palatability and chemical defenses: optimal defense theory versus the growth-differentiation balance hypothesis. *Oecologia*, 105(3):361–368.

Dancey, C. P. and Reidy, J. (2004). *Statistics Without Maths for Psychology*. Pearson.

Darling, E. S., Alvarez-Filip, L., Oliver, T. A., McClanahan, T. R., and Côté, I. M. (2012). Evaluating life-history strategies of reef corals from species traits. *Ecology Letters*, 15(12):1378–1386.

Darling, E. S., McClanahan, T. R., and Côté, I. M. (2013). Life histories predict coral community disassembly under multiple stressors. *Global Change Biology*, 19(6):1930–1940.

De'Ath, G., Fabricius, K. E., Sweatman, H., and Puotinen, M. (2012). The 27-year decline of coral cover on the Great Barrier Reef and its causes. *Proceedings of the National Academy of Sciences*, 109(44):17995–17999.

Dinsdale, E. A., Pantos, O., Smriga, S., Edwards, R. A., Angly, F., Wegley, L., Hatay, M., Hall, D., Brown, E., Haynes, M., Krause, L., Sala, E., Sandin, S. A., Thurber, R. V., Willis, B. L., Azam, F., Knowlton, N., and Rohwer, F. (2008). Microbial ecology of four coral atolls in the Northern Line Islands. *PLoS One*, 3(2):e1584–1.

Done, T. J. (1992). Phase shifts in coral reef communities and their ecological significance. *The Ecology of Mangrove and Related Ecosystems*, 13:121–132.

Doty, M. S. (1961). *Acanthophora*, a possible invader of the marine flora of Hawaii. *Pacific Science*, 15:547–552.

Duffy, J. E. and Hay, M. E. (1990). Seaweed adaptations to herbivory. *Bioscience*, 40(5):368–375.

Engel, S., Puglisi, M. P., Jensen, P. R., and Fenical, W. (2006). Antimicrobial activities of extracts from tropical Atlantic marine plants against marine pathogens and saprophytes. *Marine Biology*, 149(5):991–1002.

Erickson, A. A., Paul, V. J., Van Alstyne, K. L., and Kwiatkowski, L. M. (2006). Palatability of macroalgae that use different types of chemical defenses. *Journal of Chemical Ecology*, 32:1883–1895.

Fong, P. and Paul, V. (2011). Coral Reef Algae. In Dubinsky, Z. and Stambler, N., editors, *Coral Reefs An Ecosystem in Transition*. Springer Netherlands, Dordrecht.

Foster, N. L., Box, S. J., and Mumby, P. J. (2008). Competitive effects of macroalgae on the fecundity of the reef-building coral *Montastraea annularis*. *Marine Ecology Progress Series*, 367:143–152.

Friedlander, A., Aeby, G., Brainard, R., Brown, E., Chaston, K., Clark, A., McGowan, P., Montgomery, T., Walsh, W., Williams, I., and W, W. (2008). The state of coral reef ecosystems of the main Hawaiian Islands. In *The State of Coral Reef Ecosystems of the United States and Pacific Freely Associated States*, pages 222–269. National Oceanic and Administrative Administration.

Gardner, T. A., Cote, I. M., Gill, J. A., Grant, A., and Watkinson, A. R. (2003). Long-term region-wide declines in Caribbean corals. *Science*, 301(5635):958–960.

Godwin, S., Rodgers, K., and Jokiel, P. L. (2006). Reducing potential impact of invasive marine species in the Northwestern Hawaiian Islands marine national monument. *Report to Northwestern Hawaiian Islands Marine National Monument Administration*.

Gross, E. M. (2003). Allelopathy of aquatic autotrophs. *Critical Reviews in Plant Sciences*, 22(3-4):313–339.

Grottoli, A. G., Rodrigues, L. J., and Juarez, C. (2004). Lipids and stable carbon isotopes in two species of Hawaiian corals, *Porites compressa* and *Montipora verrucosa*, following a bleaching event. *Marine Biology*, 145(3):621–631.

Haas, A., el Zibdah, M., and Wild, C. (2009). Seasonal monitoring of coral–algae interactions in fringing reefs of the Gulf of Aqaba, Northern Red Sea. *Coral Reefs*, 29(1):93–103.

Hatcher, B. G. and Larkum, A. W. D. (1983). An experimental analysis of the factors controlling the standing crop of the epilithic algal community on a coral reef. *Journal of Experimental Marine Biology and Ecology*, 69:61–84.

Hauri, C., Fabricius, K. E., Schaffelke, B., and Humphrey, C. (2010). Chemical and physical environmental conditions underneath mat- and canopy-forming macroalgae, and their effects on understorey corals. *PLoS One*, 5(9):e12685.

Hay, M. E. (1997). The ecology and evolution of seaweed-herbivore interactions on coral reefs. *Coral Reefs*, 16(1):S67–S76.

Hay, M. E. and Fenical, W. (1988). Marine plant-herbivore interactions: the ecology of chemical defense. *Annual Review of Ecology and Systematics*, 19:111–145.

Helmuth, B., Timmerman, B. E. H., and Sebens, K. P. (1997). Interplay of host morphology and symbiont microhabitat in coral aggregations. *Marine Biology*, 130(1-10).

Horhorn, T., Hornik, K., van de Wiel, M. A., and Zeileis, A. (2008). Implementing a class of permutation tests: the coin package. *Journal of Statistical Software*, 28(8):1–23.

Hughes, T. P. (1989). Community structure and diversity of coral reefs: The role of history. *Ecology*, 70(1):275–279.

Hughes, T. P. (1994). Catastrophes, phase shifts, and large-scale degradation of a Caribbean coral reef. *Science*, 265(5178):1547–1551.

Hughes, T. P. (2003). Climate change, human impacts, and the resilience of coral reefs. *Science*, 301(5635):929–933.

Hughes, T. P., Rodrigues, M. J., Bellwood, D. R., Ceccarelli, D., Hoegh-Guldberg, O., McCook, L., Moltschaniwskyj, N., Pratchett, M. S., Steneck, R. S., and Willis, B. (2007). Phase shifts, herbivory, and the resilience of coral reefs to climate change. *Current Biology*, 17(4):360–365.

Hunter, C. L. and Evans, C. W. (1995). Coral reefs in Kaneohe Bay, Hawaii: two centuries of western influence and two decades of data. *Bulletin of marine science*, 57(2):501–515.

Jaeger, B. (2017). r2glmm: Computes R squared for mixed (multilevel) models.

Jeffrey, S. W. and Welschmeyer, N. A. (1997). Spectrophotometric and fluorometric equations in common use in oceanography. In Jeffrey, S. W., Mantoura, R. F. C., and Wright, S. W., editors, *Phytoplankton Pigments in Oceanography Guidelines to Modern Methods*. unesdoc.unesco.org, Paris, Fance.

Johnston, E. C., Forsman, Z. H., Flot, J.-F., Schmidt-Roach, S., Pinzón, J. H., Knapp, I. S. S., and Toonen, R. J. (2017). A genomic glance through the fog of plasticity and diversification in *Pocillopora*. *Scientific Reports*, 7(1):5991.

Johnston, I. S. and Rohwer, F. (2007). Microbial landscapes on the outer tissue surfaces of the reef-building coral *Porites compressa*. *Coral Reefs*, 26(2):375–383.

Jokiel, P. L., Maragos, J. E., and Franzisket, L. (1978). Coral growth: buoyant weight technique. In Stoddart, D. R. and Johannes, R. E., editors, *Coral reefs research methods*, pages 529–541. UNESCO, Paris, France.

Jompa, J. and McCook, L. (2003). Coral-algal competition: macroalgae with different properties have different effects on corals. *Marine Ecology Progress Series*, 258:87–95.

Karez, R., Engelbert, S., and Sommer, U. (2000). "Co-consumption" and "protective coating": Two new proposed effects of epiphytes on their macroalgal hosts in mesograzer-epiphyte-host interactions. *Marine Ecology Progress Series*, 2-5:85–93.

Kay, A. E. and Palumbi, S. R. (1987). Endemism and evolution in Hawaiian marine invertebrates. *Trends in Ecology & Evolution*, 2(7):183–186.

Kittinger, J. N., Bambico, T. M., Minton, D., Miller, A., Mejia, M., Kalei, N., Wong, B., and Glazier, E. W. (2016). Restoring ecosystems, restoring community: socioeconomic and cultural dimensions of a community-based coral reef restoration project. *Regional Environmental Change*, 16(2):301–313.

Kline, D. I., Kuntz, N. M., Breitbart, M., Knowlton, N., and Rohwer, F. (2006). Role of elevated organic carbon levels and microbial activity in coral mortality. *Marine Ecology*, 314:119–125.

Kvennefors, E. C. E., Sampayo, E., Ridgway, T., Barnes, A. C., and Hoegh-Guldberg, O. (2010). Bacterial communities of two ubiquitous Great Barrier Reef corals reveals both site- and species-specificity of common bacterial associates. *PLoS One*, 5:e10401.

Lapointe, B. E. (1997). Nutrient thresholds for bottom-up control of macroalgal blooms and coral reefs. *Limnography and Oceanography*, 42(5):1119–1131.

Lecky, J. H. (2016). *Ecosystem vulnerability and mapping cumulative impacts on Hawaiian reefs*. PhD thesis, University of Hawaii at Manoa.

Ledlie, M. H., Graham, N. A. J., Bythell, J. C., Wilson, S. K., Jennings, S., Polunin, N., and Hardcastle, J. (2007). Phase shifts and the role of herbivory in the resilience of coral reefs. *Coral Reefs*, 26(3):641–653.

Lesser, M. P., Bythell, J. C., Gates, R. D., Johnstone, R. W., and Hoegh-Guldberg, O. (2007). Are infectious diseases really killing corals? Alternative interpretations of the experimental and ecological data. *Journal of Experimental Marine Biology and Ecology*, 346(1-2):36–44.

Lesser, M. P. and Slattery, M. (2011). Phase shift to algal dominated communities at mesophotic depths associated with lionfish (*Pterois volitans*) invasion on a Bahamian coral reef. *Biological Invasions*, 13:1855–1868.

Lirman, D. (2001). Competition between macroalgae and corals: effects of herbivore exclusion and increased algal biomass on coral survivorship and growth. *Coral Reefs*, 19(4):392–399.

Littler, M. M. and Littler, D. S. (1984). Models of tropical reef biogenesis: the contribution of algae. In Round, F. E. and Chapman, D. J., editors, *Progress in Phycological Research*, volume 3, pages 323–364. Biopress, Bristol.

Littler, M. M. and Littler, D. S. (2007). Assessment of coral reefs using herbivory/nutrient assays and indicator groups of benthic primary producers: a critical synthesis, proposed protocols, and critique of management strategies. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 17(2):195–215.

Littler, M. M., Littler, D. S., and Brooks, B. L. (2006). Harmful algae on tropical coral reefs: Bottom-up eutrophication and top-down herbivory. *Harmful Algae*, 5(5):565–585.

Littler, M. M., Littler, D. S., and Lapointe, B. E. (1993). Modification of tropical reef community structure due to cultural eutrophication: the southwest coast of Martinique. *Proceedings of the Seventh International Coral Reef Symposium*, 1:335–343.

Longo, G. O. and Hay, M. E. (2015). Does seaweed–coral competition make seaweeds more palatable? *Coral Reefs*, 34:87–96.

Maliao, R. J., Turingan, R. G., and Lin, J. (2008). Phase-shift in coral reef communities in the Florida Keys National Marine Sanctuary (FKNMS), USA. *Marine Biology*, 154(5):841–853.

Martinez, J. (2012). *The physical and physiological impacts of the invasive red macroalga* Gracilaria Salicornia *Dawson to coral reef habitat quality and coral health*. PhD thesis, ProQuest LLC, Ann Arbor, MI.

Martinez, J., Smith, C., and Richmond, R. (2012). Invasive algal mats degrade coral reef physical habitat quality. *Estuarine, Coastal and Shelf Science*, 99:42–49.

McCook, L., Jompa, J., and Diaz-Pulido, G. (2001). Competition between corals and algae on coral reefs: a review of evidence and mechanisms. *Coral Reefs*, 19(4):400–417.

McCook, L. J. (1999). Macroalgae, nutrients and phase shifts on coral reefs: scientific issues and management consequences for the Great Barrier Reef. *Coral Reefs*, 18(4):357–367.

McManus, J. W., Menez, L. A. B., Kesner-Reyes, K. N., Vergara, S. G., and Ablan, M. C. (2000). Coral reef fishing and coral-algal phase shifts: implications for global reef status. *ICES Journal of Marine Science*, 57:572–578.

McManus, J. W. and Polsenberg, J. F. (2004). Coral–algal phase shifts on coral reefs: ecological and environmental aspects. *Progress in Oceanography*, 60(2):263–279.

Morrow, K., Ritson-Williams, R., Ross, C., Liles, M., and Paul, V. J. (2012a). Macroalgal extracts induce bacterial assemblage shifts and sublethal tissue stress in Caribbean corals. *PLoS One*, 7(9):e44859.

Morrow, K. M., Moss, A. G., Chadwick, N. E., and Liles, M. R. (2012b). Bacterial associates of two Caribbean coral species reveal species-specific distribution and geographic variability. *Applied and Environmental Microbiology*, 78(18):6438–6449.

Morrow, K. M., Paul, V. J., Liles, M. R., and Chadwick, N. E. (2011). Allelochemicals produced by Caribbean macroalgae and cyanobacteria have species-specific effects on reef coral microorganisms. *Coral Reefs*, 30(2):309–320.

Mumby, P. J. and Steneck, R. S. (2011). The resilience of coral reefs and its implications for reef management. In Dubinsky, Z. and Stambler, N., editors, *Coral Reefs: An Ecosystem in Transition*, pages 509–519. Springer Netherlands, Dordrecht.

Mumby, P. J., Steneck, R. S., Adjeroud, M., and Arnold, S. N. (2015). High resilience masks underlying sensitivity to algal phase shifts of Pacific coral reefs. *Oikos*, 125(5):644–655.

Muscatine, L. and Cernichiari, E. (1969). Assimilation of photosynthetic products of zooxanthellae by a reef coral. *The Biological Bulletin*, 137(3):506–523.

Nelson, T. A., Lee, D. J., and Smith, B. C. (2003). Are "green tides" harmful algal blooms? Toxic properteis of water-soluble extracts from two bloom-forming macroalgae, *Ulva fenestrata* and *Ulvaria obscura* (Ulvophyceae). *Journal of phycology*, 39(5):874–879.

Nugues, M. M. and Bak, R. (2006). Differential competitive abilities between Caribbean coral species and a brown alga: a year of experiments and a long-term perspective. *Marine Ecology Progress Series*, 315:75–86.

Nugues, M. M., Smith, G. W., Hooidonk, R. J., Seabra, M. I., and Bak, R. P. (2004). Algal contact as a trigger for coral disease. *Ecology Letters*, 7(10):919–923.

Odum, H. T. and Odum, E. P. (1955). Trophic structure and productivity of a windward coral reef community on Eniwetok Atoll. *Ecological Monographs*, 25(3):291–320.

Pandolfi, J. M. (2003). Global trajectories of the long-term decline of coral reef ecosystems. *Science*, 301(5635):955–958.

Pandolfi, J. M., Jackson, J. B., Baron, N., Bradbury, R. H., Guzman, H. M., Hughes, T. P., Kappel, C. V., Micheli, F., Ogden, J. C., and Possingham, H. P. (2005). Are U. S. coral reefs on the slippery slope to slime? *Science*, 307(5716):1725–1726.

Paul, V. J., Kuffner, I. B., Walters, L. J., Ritson-Williams, R., Beach, K. S., and Becerro, M. (2011). Chemically mediated interactions between macroalgae *Dictyota spp*. and multiple life-history stages of the coral *Porites astreoides*. *Marine Ecology Progress Series*, 426:161–170.

Potin, P., Bouarab, K., Salaün, J.-P., Pohnert, G., and Kloareg, B. (2002). Biotic interactions of marine algae. *Current Opinion in Plant Biology*, 5(4):308–317.

Puglisi, M. P., Engel, S., Jensen, P. R., and Fenical, W. (2007). Antimicrobial activities of extracts from Indo-Pacific marine plants against marine pathogens and saprophytes. *Marine Biology*, 150(4):531–540.

Puglisi, M. P., Sneed, J. M., Sharp, K. H., Ritson-Williams, R., and Paul, V. J. (2014). Marine chemical ecology in benthic environments. *Natural Product Reports*, 31:1510–1553.

Rasher, D. B. and Hay, M. E. (2010). Seaweed allelopathy degrades the resilience and function of coral reefs. *Communicative & integrative biology*, 3(6):564–566.

Rasher, D. B., Hay, M. E., and Ricklefs, R. (2010). Chemically rich seaweeds poison corals when not controlled by herbivores. *Proceedings of the National Academy of Sciences*, 107(21):9683–9688.

Rasher, D. B., Stout, E. P., Engel, S., Kubanek, J., and Hay, M. E. (2011). Macroalgal terpenes function as allelopathic agents against reef corals. *Proceedings of the National Academy of Sciences*, 108(43):17726–17731.

Reshef, L., Koren, O., Loya, Y., Zilber-Rosenberg, I., and Rosenberg, E. (2006). The coral probiotic hypothesis. *Environmental Microbiology*, 8(12):2068–2073.

Ritchie, K. B. (2006). Regulation of microbial populations by coral surface mucus and mucus-associated bacteria. *Marine Ecology Progress Series*, 322:1–14.

Rodgers, K. S., Jokiel, P. L., Bird, C. E., and Brown, E. K. (2010). Quantifying the condition of Hawaiian coral reefs. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 20(1):93–105.

Rodgers, S. K. and Cox, E. F. (1999). Rate of spread of introduced rhydophytes *Kappaphycus alvarezii, Kappaphycus striatum*, and *Gracilaria salicornia* and their current distributions in Kaneohe Bay, Oahu, Hawaii. *Pacific Science*, 53:232–241.

Rodrigues, L. J. and Grottoli, A. G. (2006). Lipids and chlorophyll in bleached and recovering *Montipora capitata* from Hawaii: An experimental approach. *Proceedings of the International Coral Reef Symposium*, pages 696–701.

Rodrigues, L. J. and Grottoli, A. G. (2007). Energy reserves and metabolism as indicators of coral recovery from bleaching. *Limnology and Oceanography*, 52(5):1874–1882.

Roff, G. and Mumby, P. J. (2012). Global disparity in the resilience of coral reefs. *Trends in Ecology & Evolution*, 27(7):404–413.

Rohwer, F., Seguritan, V., Azam, F., and Knowlton, N. (2002). Diversity and distribution of coral-associated bacteria. *Marine Ecology Progress Series*, 243:1–10.

Rohwer, K. and Kelley, S. (2004). Culture-independent analyses of coral-associated microbes. In Rosenberg, E. and Loya, Y., editors, *Coral Health and Disease*, pages 265–277. Springer Berlin Heidelberg.

Rosenberg, E., Koren, O., Reshef, L., Efrony, R., and Zilber-Rosenberg, I. (2007). The role of microorganisms in coral health, disease and evolution. *Nature Reviews Microbiology*, 5(5):355–362.

Russel, D. and Balaz, G. (1994). Colonization of the alien marine alga *Hypnea musciformis* (Wulfen) J. Ag. (Rhodophyta:Gigartinales) in the Hawaiian Islands and its utilization by the green sea turtule,*Chelonia mydas*. *Aquatic Botany*, pages 53–60.

Russel, D. J. (1983). Ecology of the imported red seaweed *Eucheuma striatum* Schmitz on Coconut Island, O'ahu, Hawai'i. *Pacific Science*, 37:87–107.

Sandin, S. A., Smith, J. E., DeMartini, E. E., Dinsdale, E. A., Donner, S. D., Friedlander, A. M., Konotchick, T., Malay, M., Maragos, J. E., Obura, D., Pantos, O., Paulay, G., Richie, M., Rohwer, F., Schroeder, R. E., Walsh, S., Jackson, J. B. C., Knowlton, N., and Sala, E. (2008). Baselines and degradation of coral reefs in the Northern Line Islands. *PLoS One*, 3(2):e1548–11.

Schaalie, G. B., McBride, J. B., and Fellingham, G. W. (2002). Adequacy of approximations to distributions of test statistics in complex mixed linear models. *Regional Environmental Change*, 7:512.

Schmitt, T. M., Hay, M. E., and Lindquist, N. (1995). Constraints on chemically mediated coevolution: multiple functions for seaweed secondary metabolites. *Ecology*, 76(1):107–123.

Sergeeva, O. S., Titlyanova, T. V., and Titlyanov, E. A. (2007). Species composition and distribution of algae on the fringing coral reef of Sesoko Island (Ryukyu Archipelago) before and after the natural catastrophe of 1998. *Russian Journal of Marine Biology*, 33:30–42.

Siebeck, U. E., Marshall, N. J., Klüter, A., and Hoegh-Guldberg, O. (2006). Monitoring coral bleaching using a colour reference card. *Coral Reefs*, 25(3):453–460.

Smith, J., Shaw, M., Edwards, R., Obura, D., Pantos, O., Sala, E., Sandin, S., Smriga, S., Hatay, M., and Rohwer, F. (2006). Indirect effects of algae on coral: algae-mediated, microbe-induced coral mortality. *Ecology Letters*, 9(7):835–845.

Smith, J., Smith, C., and Hunter, C. (2001). An experimental analysis of the effects of herbivory and nutrient enrichment on benthic community dynamics on a Hawaiian reef. *Coral Reefs*, 19(4):332–342.

Smith, J. E. (2003). *Factors influencing the formation of algal blooms on tropical reefs with an emphasis on nutrients, herbivores, and invasive species*. PhD thesis, University of Hawaii, Manoa.

Smith, J. E., Hunter, C. L., Conklin, E. J., Most, R., Sauvage, T., Squair, C., and Smith, C. M. (2004). Ecology of the invasive red alga *Gracilaria salicornia* (Rhodophyta) on O'ahu, Hawai'i. *Pacific Science*, 58(2):325–343.

Smith, J. E., Hunter, C. L., and Smith, C. M. (2002). Distribution and reproductive characteristics of nonindigenous and invasive marine algae in the Hawaiian islands. *Pacific Science*, 56(3):299–315.

Smith, J. E., Hunter, C. L., and Smith, C. M. (2010). The effects of top–down versus bottom–up control on benthic coral reef community structure. *Oecologia*, 163(2):497–507.

Smith, S. V., Kimmerer, W. J., Laws, E. A., and Brock, R. E. (1981). Kaneohe Bay sewage diversion experiment: perspectives on ecosystem responses to nutritional perturbation. *Pacific Science*, 35(4).

Stamski, R. E. and Field, M. E. (2006). Characterization of sediment trapped by macroalgae on a Hawaiian reef flat. *Estuarine, Coastal and Shelf Science*, 66:211–216.

Steinberg, P. D., de Nys, R., and Kjelleberg, S. (2002). Chemical cues for surface colonization. *Journal of Chemical Ecology*, 28(10):1935–1951.

Steneck, R. S. and Sala, E. (2005). Large marine carnivores: Trophic cascades and top-down controls in coastal ecosystems past and present. In *Large Carivores and the Conservation of Biodiversity*, pages 110–137. Covello.

Steven, A. and Larkum, W. D. (1993). Encore: The effects of nutrients on coral reefs. *Search*, 24:216–220.

Stimson, J. and Conklin, E. (2008). Potential reversal of a phase shift: the rapid decrease in the cover of the invasive green macroalga *Dictyosphaeria cavernosa* Forsskål on coral reefs in Kāne'ohe Bay, Oahu, Hawai'i. *Coral Reefs*, 27(4):717–726.

Stimson, J., Larned, S., and Conklin, E. (2001). Effects of herbivory, nutrient levels, and introduced algae on the distribution and abundance of the invasive macroalga *Dictyosphaeria cavernosa* in Kaneohe Bay, Hawaii. *Coral Reefs*, 19(4):343–357.

Stimson, J. S. (1987). Location, quantity and rate of change in quantity of lipids in tissue of Hawaiian hermatypic corals. *Bulletin of marine science*, 41(3):889–904.

Sweatman, H., Delean, S., and Syms, C. (2011). Assessing loss of coral cover on Australia's Great Barrier Reef over two decades, with implications for longer-term trends. *Coral Reefs*, 30(2):521–531.

Sweet, M. J., Bythell, J. C., and Nugues, M. M. (2013). Algae as reservoirs for coral pathogens. *PLoS One*, 8(7):e69717.

Tabachnick, B. and Fidell, L. (1994). *Using Multivariate Statistics*. Pearson, London, England.

Tanner, J. E. (1995). Competition between scleractinian corals and macroalgae: an experimental investigation of coral growth, survival and reproduction. *Journal of Experimental Marine Biology and Ecology*, 190(2):151–168.

Team, R. C. (2016). R: a language and environment for statistical computing.

Titlyanov, E. A., Titlyanova, T. V., Yakovleva, I. M., Nakano, Y., and Bhagooli, R. (2005). Regeneration of artificial injuries on scleractinian corals and coral/algal competition for newly formed substrate. *Journal of Experimental Marine Biology and Ecology*, 323:27–42.

Titlyanov, E. A., Yakovleva, I. M., and Titlyanova, T. V. (2007). Interaction between benthic algae (*Lyngbya bouillonii, Dictyota dichotoma*) and scleractinian coral *Porites lutea* in direct contact. *Journal of Experimental Marine Biology and Ecology*, 342(2):282–291.

Unabia, K. (1984). *Grazing of the seagrass* Halophila hawaiiana *by the snail* Smaragdia bryanae. PhD thesis, University of Hawaii at Manoa.

Walters, L. J., Smith, C. M., and Hadfield, M. G. (2003). Recruitment of sessile marine invertebrates on Hawaiian macrophytes: do pre-settlement or post-settlement processes keep plants free from fouling? *Bulletin of marine science*, 72(3):813–839.

Wedding, L. M., Lecky, J., Gove, J. M., Walecka, H. R., Donovan, M. K., Williams, G. J., Jouffray, J. B., Crowder, L. B., Erickson, A., Falinski, K., Friedlander, A. M., Kappel, C. V., Kittinger, J. N., McCoy, K., Norström, A., Nyström, M., Oleson, K. L. L., Stamoulis, K. A., White, C., and Selkoe, K. A. (2018). Advancing the integration of spatial data to map human and natural drivers on coral reefs. *PLOS One*, 13(3):e0189792.

Weijerman, M., Most, R., Wong, K., and Beavers, S. (2008). Attempt to control the invasive red alga *Acanthophora spicifera* (Rhodophyta: Ceramiales) in a Hawaiian fishpond: An assessment of removal techniques and management options. *Pacific Science*, 62(4):517–532.

Westbrook, C. E., Ringang, R. R., Cantero, S. M. A., HDAR & TNC Urchin Team, and Toonen, R. J. (2015). Survivorship and feeding preferences among size classes of outplanted sea urchins, *Tripneustes gratilla*, and possible use as biocontrol for invasive alien algae. *PeerJ*, 3(6):e1235–20.

Wolanski, E., Martinez, J. A., and Richmond, R. H. (2009). Quantifying the impact of watershed urbanization on a coral reef: Maunalua Bay, Hawaii. *Estuarine, Coastal and Shelf Science*, 84(2):259–268.

Wolanski, E., Richmond, R. H., and McCook, L. (2004). A model of the effects of land-based, human activities on the health of coral reefs in the Great Barrier Reef and in Fouha Bay, Guam, Micronesia. *Journal of Marine Systems*, 46(1):133–144.

Wommack, K. E., Ravel, J., Hill, R. T., Chun, J., and Colwell, R. R. (1999). Population dynamics of Chesapeake Bay viroplankton: total-community analysis by pulsed-field gel electrophoresis. *Applied and Environmental Microbiology*, 85:231–240.

Woo, M., Smith, C., and Smith, W. (2002). Ecological Interactions and Impacts of Invasive *Kappaphycus striatum* in Kane'ohe Bay, a Tropical Reef. *Proceedings, First National Conference of Tropical Reef Marine Bioinvasions*, 186:1–46.

Yamashiro, H., Oku, H., and Onaga, K. (2005). Effect of bleaching on lipid content and composition of Okinawan corals. *Fisheries Science*, 71:448–453.